The role of cell savers and filters in cardiac surgery

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Chapter 1

General introduction

General introduction

Although there is widespread and increasing evidence for a lower threshold in transfusion of red blood cells, transfusions are still common in patients undergoing cardiac surgery and continue to increase [1-3]. The transfusion threshold has been steadily decreasing the past decades, but up to the beginning of 2015 no high quality study and no international consensus on the optimal haemoglobin concentration at which to transfuse in the cardiac surgical population exists [4-8]. Recently a high quality study was published that showed that a restrictive transfusion threshold was not superior to a liberal transfusion threshold [9]. In the peri-operative care for cardiac surgical patients, transfusion guidelines are formed on a national or local level, but personal physician beliefs still play a large role in a significant proportion of the prescribed transfusions [10]. Thus, transfusion thresholds vary widely between physicians, hospitals and countries [11,12].

There is ample evidence, mainly based on physiological findings, for the use of red blood cell transfusions to improve oxygen delivery to tissues in states of haemorrhage and/or anaemia [1,2,4,5]. The WHO defines anaemia as a hemoglobin (Hb) concentration below 8.1 mmol/l for men and below 7.4 mmol/l for women. Its presence is associated with increased intensive care and hospital length of stay and increased mortality in cardiac surgery [13,14]. Intra-operative anaemia may be the cause of post-operative kidney injury requiring renal replacement therapy and it is also associated with a prolonged intensive care unit stay and a higher mortality rate [15].

The negative effects of blood transfusions are on the other hand well known. Blood transfusions have been described to increase morbidity and mortality in patients undergoing or after cardiac surgery [16-20]. Transfusion of blood and blood products may cause lung injury, increase the incidence of post-operative pneumonia, and prolong post-operative mechanical ventilation and thus the length of intensive care stay [16,18,21,22]. Furthermore, allogeneic blood transfusions are associated with increased post-operative infections, including sternal wound infections and sepsis [23,24]. Sternal wound infections in turn have a strong association with the number of re-thoracotomies and persistent bleeding is a common cause for a re-thoracotomy. In light of this evidence, a reduction of peri-operative blood transfusion is desirable and important for both patient and the health care provider.

Blood transfusion sparing strategies

Not surprisingly, a lot of effort has been put in means to decrease the number of red blood transfusions in cardiac surgery by publishing multi-disciplinary guidelines for clinical practice in blood sparing strategies. These can generally be divided in to pre-, intra- and post-operative strategies [1,2,25-28].

Pre-operative blood conservation strategies include patient risk assessment, management of drugs that interfere with coagulation (i.e. cessation of acetylsalicylic acid, P2Y12 ADP receptor blockers, coumarins), adequate diagnosis and therapy of pre-operative anaemia.

Intra-operative blood conservation strategies can be divided into pharmacological interventions (i.e. the use of anti-fibrinolytic and pro-haemostatic drugs), the prevention of haemodilution (i.e. a restrictive fluid management), adequate monitoring of blood coagulation and the application of restrictive transfusion triggers. Further actions can be taken while the patient is on cardiopulmonary bypass (CPB) (i.e. use of biocompatible coatings, performing off-pump surgery, maintaining normothermia) and the use of cardiotomy suction and/or cell salvers. Post-operative blood conservation strategies are essentially the same as the intra-operative strategies, including the use of cell savers.

Although much effort has been put into providing physicians with evidence-based guidelines on blood sparing strategies, there is still much debate about the efficacy of many of these recommendations [3]. This is mainly because the levels of evidence on which guidelines are based are often low and large randomized controlled clinical trials have not been performed yet. In addition, there is a plethora of small studies on blood sparing strategies with different and sometimes contradicting results. Unfortunately there is also the difficulty in changing longstanding transfusion practices, a ”low and slow adoption rate”, on the practitioners level [3,10]. Without strong evidence changing clinical practice and personal attitudes is difficult.

Focus on cardiotomy as blood sparing strategy

As mentioned cardiotomy suction can be used as a strategy to reduce blood loss during cardiac surgery and hence reduce the need for allogeneic blood transfusions. The cardiotomy suction was introduced in the 1960s as an extension of the intra-

cardiac vent. During CPB blood from the surgical field together with other contents of the pericardial cavity, is aspirated into a cardiotomy reservoir. After passing through the oxygenator the blood is returned to the patient instead of discarding it. Although this strategy is considered standard practice in cardiac on-pump surgery and its use is especially justified in the presence of rapid significant blood loss there is surprising little to no evidence for cardiotomy suction use during CPB in routine cardiac surgery as a blood conserving strategy [2,29].

Cardiotomy blood quality

Besides the lack of evidence for the use of cardiotomy suction in routine cardiac surgical procedures, there are also disadvantages with the use of cardiotomy suction. The cardiotomy blood differs markedly from intravascular blood or blood within a closed CPB circuit with respect to altered coagulation, induction of inflammation, formation of micro-particles and induction of haemolysis.

Exposure of the shed blood to tissue factor results in activation of the coagulation system with release of thrombin and fibrin. In addition, tissue plasminogen activator released from the surgical trauma stimulates fibrinolysis. Analysis of pericardial shed blood shows high concentrations of markers of both clotting activation and fibrinolysis. Furthermore platelets are activated during extravasation of blood into the pericardial cavity. This results in aggregation, degranulation and consumption of platelets, as well as the release of inflammatory markers (for example interleukin-6). [30-33]. Activation of the coagulation cascade inevitably results in co-activation of inflammatory cascades [29,34]. As a consequence, pericardial suction blood contains high concentration of pro-inflammatory cytokines and as such retransfusion of cardiotomy blood may contribute to the inflammatory response associated with cardiac surgery and can impair haemodynamics, renal and pulmonary function [29-31,35-37].

The pericardial space also contains a mixture of organic and non-organic debris such as sternal marrowfat, aggregated platelets and fragments of bone wax. Furthermore, there is mixing of air and blood with subsequent formation of micro-particles when blood is aspirated to the cardiotomy reservoir [38,39]. Neurocognitive dysfunction after cardiac surgery is associated with retransfusion of cardiotomy suction blood and is one of the main reasons for decreased quality of life post-operatively [40,41]. Micro-

particles can cause micro-embolisms in other organs, such as the kidneys and impair their function [36,42]. Cardiotomy suction is additionally associated as one of the main sources of haemolysis [35,43,44]. A complete avoidance of cardiotomy suction would probably decrease not only micro-emboli, but also the inflammatory reaction and haemolysis [29,34,35].

In summary, cardiotomy suction blood is far from clean and contains a substantial fraction of potential embolic substances, haemolytic blood, activated platelets and pro-inflammatory substances that can impair haemostasis and increase the inflammatory response. This should at least lead to re-evaluating the routine use of cardiotomy suction during low risk cardiac surgical operations in which there is no anticipated large amount of intra-operative blood loss, such as primary aortic valve replacement or coronary artery bypass grafting (CABG). In an effort to ameliorate some of these potentially detrimental effects of cardiotomy suction blood, strategies were developed for specific lipid, red blood cell and leukocyte filtration while still being able to recuperate red blood cells. The anticipation was that application of these alternative strategies would reduce or ideally remove the pro-embolic, pro-inflammatory, pro-fibrinolytic and pro-coagulatory elements of the cardiotomy suction blood.

Role of leukocyte depletion filters in improving the cardiotomy blood quality

One approach to improve the quality of cardiotomy suction blood is to eliminate or reduce micro-embolization. This can be achieved by the application of fat or leukocyte depletion filters. Fat filters can actually decrease the incidence of embolization, but they do not have an effect on the pro-inflammatory reaction [39,45-47]. Leukocyte depletion filters in turn might improve the blood quality by the removal of activated leukocytes but also fat and other particles from the collected blood. Activated leukocytes play a major role in the initiation of a systemic inflammatory response and organ reperfusion injury in patients undergoing cardiac operations with cardiopulmonary bypass [48-50]. In recent decades, the concept of leukocyte depletion has been introduced to cardiac surgery to remove leukocytes by means of various strategies. These include arterial line filtration, removal from the local blood cardioplegia circuit, during the reperfusion phase on CPB, from banked blood or from the salvaged blood and residual heart-lung

machine blood [51]. Furthermore filtration of retransfused blood salvaged by a cell saver device has also been applied [52-55]. In theory, if blood from the cardiotomy suctions reservoir is retransfused to the patient through a leukocyte depletion filter, the release of pro-inflammatory agents is reduced and the plasma fraction of the blood is retained (in stead of lost when for example a cell saver is used) [56]. However, although leukocyte depletion is often reported as a successful method in reducing inflammation, nearly all of the clinical trials have failed to demonstrate clinical benefits on overall patient outcomes such as morbidity, mortality, and hospital stay [50-52,57-59]. Possible reasons why existing studies did not find a positive effect of the filters are the small number of patients in the existing studies, poor quality of the trials (non-randomized), different patient populations and focus on laboratory effects instead of clinical endpoints. Furthermore the studies varied in the application of leukocyte filtration regarding the timing (during cross-clamp and/or during reperfusion), duration of filtration (intermittent versus continuous), regarding the position of the filters (arterial and/or cardioplegic line), frequency of filter change due to filter capacity exhaustion and to a lesser extent the type of filter used [51,59]. But there is some evidence that points to specific patient subgroups that could benefit from leukocyte depletion. These are patients with chronic obstructive pulmonary disease, high-risk patient groups (left ventricular hypertrophy, reduced left ventricular ejection fraction, cardiogenic shock) and high-risk procedures (coronary bypass and valve surgery with cross clamp times greater then 120 minutes, paediatric cardiac surgery, emergency coronary artery bypass or cardiac transplantations) [58,60]. But, again, recent studies with leukocyte depletions in these specific patient groups and a Cochrane review did not show an significant improvement in clinical outcomes [49,61-63]. The method of leukocyte filters on the CPB circuit is not recommended in the current guidelines [1].

Role of cell savers in improving cardiotomy blood quality and as blood sparing strategy

Cell savers are used in cardiac surgery to recover intra-operative shed blood in order to reduce the need for allogeneic blood transfusions. The cell saver was introduced by the end of the 70's in the past century in the United States as a means to bypass the cardiotomy suction in cardiac surgery. One of the first randomized controlled trial with an intra-operative cell saver was performed in 1993 in the United States [64]. This

small study showed that intra-operative use of a cell saver as alternative of cardiotomy suction decreased the exposure to allogeneic blood transfusions in comparison to discarding the peri-operative shed blood. At the end of the 20th century the use was expanded to Europe when the Health Services Circular "Better Blood Transfusion" in Great Britain advocated its use [65,66].

The process of intra-operative cell salvage can be divided into three phases: blood collection, blood washing, and re-transfusion. Blood from the operative field is collected, together with other contents of the pericardial cavity, by the use of a double-lumen suction tube. One lumen suctions blood from the operative field and the other lumen adds a volume of heparinized saline to the salvaged blood. The blood is then passed through a filter and collected in a reservoir for processing (hence the need for anticoagulation). The blood is centrifuged and then washed with a solution. The process removes plasma, platelets, leukocytes, free hemoglobin and heparin [67]. This results in a concentrated amount (haematocrit between 50-80%) of recovered red blood cells ready to be retransfused to the patient. Contrary to the use of cardiotomy suction alone, collection of shed blood is thus possible outside the time frame of the CPB period with the use of a cell saver e.g. even before application of full dose heparin and after application of protamine. Intra-operatively used, cell saver can also process the remaining volume in the CPB circuit. Furthermore, the cell saver can be employed during the post-operative period to process shed mediastinal blood. With this approach the possibility to recover shed blood is extended.

The Society of Thoracic Surgeons and the society of Cardiovascular Anaesthesiologists have recently updated their blood conservation clinical practice guidelines. Accordingly the use of cell savers is a class I recommendation in all patients except in those with infections or malignancy [1].

The influence of processing cardiotomy suction blood with a cell saving device on the quality of the blood has been investigated in several studies. Quality aspects of collected and/or retransfused blood studied vary between free haemoglobin [44], heparin [68], platelet, leukocyte [69-71] and fat elimination [39,42,47,72,73]. Other parameters that have been extensively investigated are the effect of cell salvage on pro-inflammatory cytokines [37,74-77] and haemostasis [67,69,76-79] in the collected and patients blood. Cell salvage can result in the activation of white blood cells leading to the release of

inflammatory mediators. However, the centrifugation and the washing process reduces the concentration of these white blood cells and inflammatory mediators [75]. Transfusing blood from the cardiotomy reservoir after centrifugation leads to a decrease in systemic vascular resistance and can lead to a decreased afterload which can improve post-operative cardiac function [37].

Recently focus has shifted to the function of red blood cells processed by the cell saver in cardiac surgery. This quality aspect of blood was initially investigated in stored red blood cells. The changes that were found in these stored red blood cells were called "storage lesions" [80]. RBC function examined is the cell membrane deformability or the ability for the cell to elongate when exposed to shear stress while being forced through a thin channel [81,82]. RBC cell membrane elasticity and the ability to change shape are important qualities of the RBC's to pass through small capillaries. Impaired RBC membrane deformability is associated with reduced flow in the microcirculation, as well as reduced regional blood flow and reduced oxygen delivery [83]. RBC aggregation is an important, shear dependent, determinant of blood viscosity. RBC aggregation and deformability are affected by blood storage [84]. Moreover, RBC function can be studied by looking at the depletion of the 2,3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP) content. 2,3-DPG is necessary for regulating oxygen delivery and ATP is the energy source for the overall functioning of the red blood cell. In the limited studies on red blood cell function in cardiac surgical patients, the cell salvage process does not seem to impair the overall red blood cell function, although there appears to be a reduction in the deformability of the red blood cells. There are contradictory results on the effect of cell salvage on the 2,3-DPG content [52,79,85].

Two recent meta-analyses demonstrated a risk reduction for blood transfusion during cardiac surgery using intra-operative cell salvage [86,87]. However there was great heterogeneity between the reviewed studies. This was mainly because of the different initial objective of cell saver deployment. In studies where cell savers were predominantly used for blood conservation, a comparison was made between discarding blood before and after the CPB time period and between collecting, washing and re-transfusing this otherwise discarded blood [88,89]. However, in a number of other studies, cell savers were mainly used for organ protection and a reduction of the inflammatory response that is associated with cardiopulmonary bypass [41,53,90].

In these studies the comparison was typically made between washed and unwashed blood both of which were returned to the patient (for example cardiotomy suction blood). These different strategies likely affect the possible reduction in transfusion requirements. Studies on organ protection and cell savers were performed to try to eliminate embolic load of the cardiotomy suction blood [41,53]. Intra-operative use of cell savers can decrease the fat-related micro-embolic load significantly [39,42,45,91]. The processing of blood by a cell saver reduces fat micro-embolism to the central nervous system and can thus decrease the incidence of neurological complications after cardiac surgery procedures [41,42]. However, some published studies doubt the theoretical benefit when it comes to less complications [53].

Another reason for the heterogeneity between the reviewed studies, mainly regarding the blood transfusion data, is the different time frame period of cell saver employment in nearly all studies (table 1).

Table 1: Time frame of cell saver use in recent studies

Study	Pre- or post-CPB	CPB	Residual CPB blood	Post op
Attaran [92]	+	-u[§]	-	-
McGill [88]	+	-	+	-
Reyes [93]	+	-	+	-
Klein [89]	+	-	+	+
Murphy [94]	+	-	-	+
Damgaard [75]	+	+	+	-
Diprose [95]	+	+	+	-
Laub [64]	+	+	+	-
Weltert [96]	+u	+[¶]	+[¶]	+
Jewel [91]	u	+	+	-
Vonk [97]	-	+	+	-
Svenmarker [98]	-	+	-	-
Djaiani [41]	-	+	-	-
Westerberg [99]	-	+	-	+
Rubens [53]	-	+	-	+
Dalrymple [100]	-	-	+	+
Sirvinskas [101]	-	-	-	+

Pre-or post-CPB, use before or after cardiopulmonary bypass; CPB, use during cardiopulmonary bypass; Post-op, use during intensive care stay.
-= not used; += used; u = unclear if used; ¶= includes 35% off pump cardiac surgery; §, includes 20% off pump

This variation in cell saver deployment, as shown in table 1, is of course partly understandable a result of the difference in initial purpose of cell saver deployment: blood conservation [64,88,89,94,96,97] or organ protection [41,53]. Others have used the cell saver in order to investigate whether there is a reduction of inflammatory markers or formation of haemolytic blood [98,99]. When used in such a manner, the cell saver is typically only used during the CPB period, with some studies also processing the remaining CPB volume.

When a cell saver is mainly used as a blood transfusing sparing strategy the time period is usually the entire operation, and sometimes beyond [96,97]. Processing the cardiotomy suction blood with a cell saver during CPB only has no significant effect on blood conservation but can increase the need for fresh frozen plasma transfusion [41,86]. Some studies were a reflection of the current operating procedures in that specific institution [89,94]. Only a few authors use the cell saver to collect all shed blood during the operation, not only blood lost during CBP time [96,97]. Deployment of a cell saver can only be optimal as a blood conservation strategy when all shed blood is processed, is the stated argument. Cell savers have therefore also been used, as an extension of this argument, to process and re-transfuse post-operative shed mediastinal blood in addition to intra-operative use alone [53,89,94]. One study centered on this topic [96].

Furthermore, the amount of blood processed and thus the amount of RBC retransfused to the patient, by the cell savers varies substantially in the above mentioned studies making firm conclusions on the effect of the use of the cell saver on transfusion requirements difficult. Another methodological problem in cell saver studies is the lack of data on collected blood volume and final processed blood volume. These volumes, however, are important to calculate the crude extraction ratio and thus understand the full efficacy of the cell saver.

Despite the increasing pro arguments for the use of cell savers during cardiac surgery there are still studies that conclude that a cell saver should not routinely be used in low risk cardiac surgery and that it's use should perhaps only be implemented in medium to high-risk surgery. Use in low risk surgery is not blood sparing and not cost effective [92,93]. Alas, these studies lack methodological rigor and include a large proportion of off-pump cardiac surgery or the cell saver is not used during CPB (table 1). Two recent studies specifically show that the cell saver can also be effective in low to intermediate risk cardiac surgery [96,97].

Investigational questions

In the light of the questions raised in the discussion above we wanted to investigate whether the quality of the collected and retransfused blood in cardiac surgery could be improved. Furthermore, the question remained whether cell savers, or the use of filters, can reduce allogeneic blood transfusions in cardiac surgery. In this thesis we have tried to lift the veil on some of these matters.

Outline of the thesis

The first part of this thesis serves as an introduction into the different possibilities and difficulties in blood sparing strategies in cardiac surgery. The main focus on improving cardiotomy suction blood by filtration and use of a cell saver device is introduced. In **chapter 2** the effect of cell saving, leukocyte depletion filters and/or their combination on transfusion requirements in cardiac surgery is described. In **chapter 3** the question whether the quality of processed blood is affected when larger quantities of blood are processed in multiple intra-operative processing runs of a cell saver device is described. In **chapter 4** whether additional post-operative collection and processing of mediastinal shed blood with a cell salvage device reduces the number of allogeneic blood transfusions compared to intra-operative cell salvage alone is described. In **chapter 5** the leukocyte and fat removal properties and the biocompatibility of three different filters is described. Finally in **chapter 6** whether the use of a cell saver influence red blood cell function and if retransfused processed blood affects RBC function in patients is described. **Chapter 7** provides a summary, general conclusion and discussion on future perspectives.

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Chapter 2

Effects of cell saving devices and filters on transfusion in cardiac surgery: a multicenter randomized study

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Abstract

Background: Cell-saving devices (CS) are frequently used in cardiac surgery to reduce transfusion requirements, but convincing evidence from randomized clinical trials is missing. Filtration of salvaged blood in combination with the CS is widely used to improve the quality of retransfused blood, but there are no data to justify this approach.

Methods: To determine the contribution of CS and filters on transfusion requirements, we performed a multicenter factorial randomized clinical trial in two academic and four non-academic hospitals. Patients undergoing elective coronary, valve or combined surgical procedures were included. The primary end point was the number of allogeneic blood products transfused in each group during hospital admission.

Results: From 738 included patients, 716 patients completed the study (CS + filter: 175, CS: 189, filter: 175, neither CS nor filter: 177). There was no significant effect of CS or filter on the total number of blood products (fraction [95% confidence interval]: CS: 0.96 [0.79, 1.18]; filter: 1.17 [0.96, 1.43]). Use of a CS significantly reduced red blood cell transfusions within 24 hours (0.75 [0.61,0.92]), but not during hospital stay (0.86 [0.71, 1.05]). CS was significantly associated with increased transfusions of fresh frozen plasma (1.39 [1.04; 1.86]), but not with platelets (1.25 [0.93; 1.68]). Use of a CS significantly reduced the percentage of patients who received any transfusion (odds ratio [95%CI): 0.67 [0.49; 0.91]), whereas filters did not (0.92 [0.68, 1.25]).

Conclusion: Use of a CS, with or without filter, does not reduce the total number of allogeneic blood products, but reduces the percentage of patients who need blood products during cardiac surgery.

Introduction

There is some evidence that cell-saving devices (CS) reduce red blood cell (RBC) transfusion during cardiac surgery, but that extensive use of a CS may lead to a bleeding diathesis [1,2]. Two recent meta-analyses [3,4] suggest that fewer patients receive allogeneic blood transfusions when a CS is used. However there was substantial heterogeneity due to different blood conservation concepts of the included studies, and most studies were underpowered with methodological shortcomings. Furthermore, transfusion of higher volumes of cell saver blood is associated with increased transfusion requirements of fresh frozen plasma (FFP) [2,5]. For a valid comparison of transfusion requirements it is therefore important to consider all administered blood products and the percentage of patients transfused during hospital admission. Currently, there are no randomized clinical trials with sufficient statistical power to justify the routine use of a CS in cardiac surgery.

Many institutions nowadays use an additional filter for transfusion of CS to improve the quality of the retransfused blood. Although this is recommended by several authors and manufacturers [6-8] this practise is not supported by clinical data.

Retransfusion of cardiotomy suction blood to the cardiopulmonary bypass (CPB) circuit is usually the first step in blood conservation during cardiac surgery. Cardiotomy blood is highly inflammatory and associated with increased transfusion requirements and organ injury [9,10]. It can be processed either by washing with a CS [2] or by passing through a leukocyte depletion (LD) filter [8]. This latter approach may improve coagulation as the plasma fraction of the blood is retained. This may decrease transfusion requirements. We previously demonstrated that retransfusion of residual blood from the CPB circuit through a LD filter resulted in improved post-operative lung function [7]. Thus, transfusion of both cardiotomy suction blood and residual blood from the CPB circuit through a LD filter could have a similar clinical effect as processing this blood with a CS.

Considering the possible positive and negative effects of CS and filters on the use of allogeneic blood products during cardiac surgery and the lack of sufficient clinical evidence we conducted a multicenter factorial randomized clinical trial to investigate the effect of CS, LD filters, and their combination on transfusion requirements in cardiac surgical patients.

Methods

This study was a partially blinded randomized 2x2 factorial multicenter trial with CS and LD filter as the two factors. Adult patients scheduled for elective coronary artery bypass grafting, valve surgery or combined procedures were included. Patients scheduled for off-pump surgery and patients with known coagulation disorders except after the use of aspirin, clopidogrel or low molecular-weight heparin were excluded. Aspirin and clopidogrel were stopped according to local protocol. Each institutional review board approved the study (2 academic and 4 non-academic centers), covering the whole country (fig 1) and informed consent was obtained from all patients included in the study. Only morning scheduled patients were included.

In groups 1 (CS), 2 (CS + filter) and 3 (filter) cardiotomy suction blood, blood from the surgical field, and residual heart lung machine blood, was collected. This blood was washed with a CS in groups 1 and 2 and retransfused through a standard blood giving set in group 1, and through a LD filter in group 2 and 3. In group 4 (control) neither CS nor filters were used. Instead, conventional cardiotomy suction was used and blood from the surgical field was discarded after reversal of heparin. Residual heart lung machine blood was retransfused through a standard blood giving set.

Anaesthesia, surgery and CPB were performed according to institutional practice. Protease inhibitors were not used. The CPB circuit was primed with 1000mL lactated Ringer's solution and 500mL hydroxyethylstarch 10% (Fresenius, Bad Homburg, Germany). Pump flow was 2.4 L/m2/min and temperature was allowed to drift to 34°C. Heparin was given to obtain activated clotting time >400 seconds and was reversed with protamine in a 1:1 ratio after CPB. Hemoconcentrators were not used. The centers used their own CS with standard washing program (CATS (Fresenius, Bad Homburg, Germany), Brat 5 (Haemonetics, Braintree, MA, USA), or Dideco-electa (Sorin, Milan, Italy)). Suction pressure was minimized to prevent haemolysis. Biofil 2 LD filters (Fresenius, Bad Homburg, Germany) were used and changed after 1000 ml of blood and after 250 ml of CS-processed blood [11].

Based on Dutch transfusion guidelines RBC's were transfused when the post-operative hemoglobin level was <5 mmol/L. Transfusion of RBC's during CPB was guided by clinical judgment of the attending anaesthesiologist and perfusionist. Transfusion

of FFP occured in case of excessive bleeding (>150 ml/h for 2 consecutive hours and prothrombin time >1.5 times normal). Platelets were transfused when platelet counts were $<100x10^9$/L in combination with excessive bleeding. The decision for surgical reexploration was made on the usual clinical grounds.

Patients were extubated when normothermic, haemodynamically stable with an arterial partial pressure of oxygen of greater than 9 kPa on minimal ventilatory support. It is standard policy in the Netherlands to transfer patients after cardiac surgery to the ward in the morning.

The primary endpoint was the number of allogeneic blood products used in each group during hospital admission. Secondary end points were percentage of patients who received any allogeneic blood products, number of reexplorations, myocardial infarction, stroke, post-operative ventilation time, length of stay in the intensive care unit and in the hospital, and 1-year mortality.

Preliminary data from the institutional database of the investigational leading center (Groningen) was used to calculate sample sizes with the two-sample Student's *t*-test using PASS software, version 6.0 (NCSS, LLC, Kaysville, UT). A difference in the average value of 466 ml (control) and 274 ml (CS + filter) blood products per patient requires 150 patients in each group (α= 0.05, β= 0.80), and an overly conservative standard deviation of 593 ml. Because of the multicenter character of the study we chose to include at least 180 patients per treatment group.

For each center a computer-generated randomization table was made with four groups. Allocation was done with sealed sequentially numbered envelopes. The study was not blinded for the intra-operative part, because the CS could not be concealed by its size, noise and special suction tube. However, all other caregivers were blinded to the intervention.

The analysis was based on the intention-to-treat principle. Logistic regression was used for binary outcomes and Poisson regression for count variables. Overdispersion was estimated with the deviance statistic. Linear regression was used for concentrations and for the logarithmic transformed blood loss. These analyses included an effect of CS, of filter, and an interaction effect and they were corrected for centers, except for the outcomes mortality, myocardial infarction, and stroke. The effects of CS and filter, with their 95% confidence intervals, and the p-value for the interaction effect are

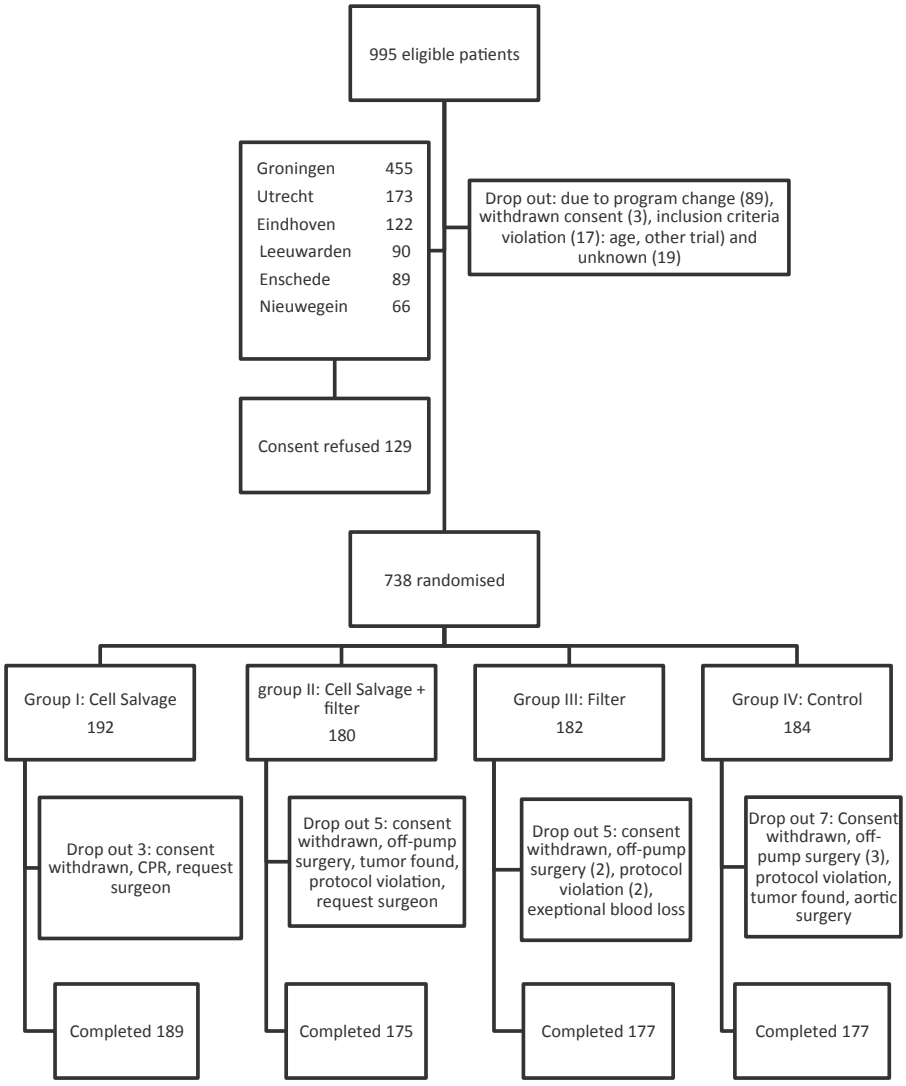
reported. For binary outcomes the odds ratio was used, for count data and blood loss the ratio of average blood products between treatment groups and for concentrations the mean difference was used. A p-value below 0.05 was considered significant. All statistical analyses were performed with SAS, version 9.3 (SAS institute Inc, Cary, NC).

Results

Participants were recruited from January 2005 to January 2009. The flowchart through the trial is shown in fig 1.

Seven hundred sixteen patients completed the study. All transfusion data were not available from 6 patients (3 in the filter group and 3 in the control group). As a result of the implementation of new Dutch transfusion guidelines during the study period tranexamic acid (2g) was used in 156 patients, equally divided over the four groups. The demographic data are shown in table 1, and the intra-operative data in table 2.

Fig 1. Flow chart of patients through the trial



CPR, cardiopulmonary resuscitation

Table 1: Demographic Data

Characteristics	CS (n= 189)	CS + Filter (n=175)	Filter (n=175)	Control (n=177)
Age (y)	66 ± 9.5	65 ± 9.7	66 ± 10.5	66 ± 9.7
Height (cm)	173 ± 8	174 ± 8	174 ± 9	172 ± 9
Weight (kg)	81 ± 13	84 ± 13	84 ± 14	81 ± 14
Male, n (%)	134 (71)	140 (80)	132 (75)	127 (71)
Euro SCORE	4.2 ± 3.0	4.3 ± 3.0	4.7 ± 3.3	4.7 ± 3.4
Myocardial infarction (%)	23	21	28	27
Hypertension (%)	46	46	39	46
Stroke (%)	4	6	7	6
Atrial fibrillation (%)	13	11	12	12
Diabetes (%)	24	22	15	21
Pulmonary disease (%)	11	14	15	10
Aspirin < 3 days (%)	37	45	42	44
Clopidogrel < 3 days (%)	7	5	4	6
Beta-blocker (%)	68	68	69	70
Calcium antagonist (%)	31	22	24	32
ACE inhibitor (%)	43	46	38	36
Haemoglobin (mmol/l)	7.6 ± 0.9	7.6 ± 0.9	7.6 ± 0.9	7.5 ± 0.9
Creatinine (mmol/l)	84 ± 19	87 ± 23	88 ± 21	90 ± 35

ACE, angiotensin-converting enzyme; CS = cell-saving device; Euro SCORE = European System for Cardiac Operative Risk Evaluation

Table 2: Intra-operative Data: Procedures and Cardiopulmonary Bypass Management[a]

Variable	CS	CS + Filter	Filter	Control
CABG, n (%)	116 (61)	106 (61)	110 (63)	115 (65)
Valve, n (%)	54 (29)	44 (25)	33 (19)	37 (21)
CABG + valve, n (%)	19 (10)	25 (14)	32 (18)	25 (14)
CPB time (min)	103 ± 41	104 ± 43	105 ± 35	104 ± 45
Cross-clamp time (min)	65 ± 27	67 ± 29	68 ± 26	68 ± 30
Hemoglobine CPB (mmol/L)	4.8 ± 0.70	4.9 ± 0.76	4.9 ± 0.79	4.8 ± 0.75
Residual CPB blood (mL)	784 ± 490	774 ± 421	815 ± 461	951 ± 472
Blood collected (mL)	1,310 ± 1,186	1,537 ± 1,541	1,463 ± 971	NA
CS processed (mL)[b]	658 ± 390	684 ± 514	NA	NA

[a] Data are presented as mean ± SD unless otherwise stated. [b] Residual CPB blood plus blood collected. CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; CS, cell-saving device; NA, not applicable.

The overall transfusion data are shown in table 3. This table also contains the effect size with the 95% confidence interval for the treatment comparisons: "use of a CS versus no use of a CS" and "use of a filter versus no use of a filter".

Table 3: Transfusion Data Overall

Variable	CS (n=189)	CS + Filter (n=175)	Filter (n=175)	Control (n=177)	Effect of CS[a]	Effect of Filter[a]	p value[b]
Total units RBC in first 24 h	205	186	255	244	0.75 (0.61-0.92)	1.02 (0.83-1.25)	0.84
Patients transfused RBC in first 24 h, n (%)	76 (40)	61 (35)	90 (52)	86 (49)	0.57 (0.42-0.78)	0.95 (0.70-1.29)	0.35
Total units RBC during hospital admission	358	355	429	357	0.86 (0.71-1.04)	1.13 (0.93-1.38)	0.63
Patients transfused with RBC during hospital admission, n (%)	94 (50)	79 (45)	103 (59)	104 (59)	0.58 (0.42-0.79)	0.92 (0.67-1.25)	0.73
Total units FFP	97	109	78	64	1.39 (1.04-1.85)	1.22 (0.91-1.62)	0.95
Patients transfused with FFP, n (%)	30 (16)	30 (17)	24 (14)	29 (16)	1.12 (0.74-1.70)	0.94 (0.62-1.43)	0.43
Total units platelets	32	51	32	30	1.24 (0.92-1.67)	1.33 (0.99-1.79)	0.09
Patients transfused with platelets, n (%)	25 (13)	33 (19)	24 (14)	22 (13)	1.25 (0.82-1.91)	1.30 (0.85-1.99)	0.43
Total units RBC, FFP, and platelets	487	515	539	451	0.96 (0.78-1.17)	1.16 (0.96-1.42)	0.92
Patients transfused with any RBC, FFP, and platelets, n (%)	98 (52)	83 (47)	103 (59)	108 (61)	0.67 (0.49-0.91)	0.91 (0.67-1.24)	0.92

[a] Effects are ratio and 95% confidence intervals. A ratio larger than 1 indicates a higher risk for blood products in the control group. [b] The p value indicates the interaction effect of the CS in combination with the filter.
CS, cell-saving device; FFP, fresh frozen plasma; RBC, red blood cells.

Use of a CS resulted in a significant reduction of 25% (95% confidence interval [CI], 8% to 39%) in the number of RBC's that were transfused within the first 24 hours. This effect decreased to 14% (95% CI,-5% to 29%) in the further post-operative period. However, there was only a 4% (95% CI, -18% to 21%) reduction in the total number of blood products that were transfused during hospital admission (table 3). In the groups with CS, 56.1% (95% CI, 49.5% to 62.4%) patients used blood products versus 65.6% (95% CI, 59.2% to

71.4%) in the groups without CS. In contrast, use of a filter was associated with higher transfusion requirements, although none of the effects were statistically significant (table 3). Transfusion data of the individual patients are presented in table 4. When a CS was used 22% of the platelets were transfused without concurrent administration of FFP and 35% when a CS was not used.

Table 4: Transfusion Data by Patient Level

Variable	CS (n=189)	CS + Filter (n=175)	Filter (n=175)	Control (n=177)
Units RBC transfused in first 24 h to patients (n)				
0	113	114	84	91
1-2	48	32	50	48
3+	28	29	40	38
Units RBC/patient in first 24h	1.1	1.1	1.4	1.4
Units RBC/transfused patient in first 24h	2.1	3.0	2.8	2.8
Units RBC transfused to patients during hospital admission (n)				
0	95	96	72	73
1-2	48	36	41	51
3+	46	43	62	53
Units RBC/patient during hospital admission	1.9	2.0	2.5	2.0
Units RBC/transfused patient during hospital admission	3.8	4.5	4.2	3.4
Units FFP transfused to patients (n)				
0	159	145	151	148
1-2	15	16	12	24
3+	15	14	12	5
Units FFP/patient	0.5	0.6	0.4	0.4
Units FFP/transfused patient	3.2	3.6	3.2	0.6
Units platelets transfused to patients (n)				
0	164	142	150	153
1	20	24	18	17
2+	5	9	6	5
Units platelets/patient	0.2	0.3	0.2	0.2
Units platelets/transfused patient	1.3	1.5	1.3	1.4

CS, cell-saving device; FFP, fresh frozen plasma; RBC, red blood cell concentrate

Post-operative data are presented in table 5 in a similar way as in table 3. For post-operative blood loss we observed an interaction effect. When the filter was applied an effect of the CS was detected (0.81; 95% CI, 0.71 to 0.94) and when no CS was used, an

effect of the filter was almost significant (1.13; 95% CI, 0.98 to 1.31). The lowest average blood loss was obtained with the use of a filter and a CS. Although all groups had similar pre-operative hemoglobin levels (table 1), and during CPB (table 2), use of a CS resulted in higher post-operative hemoglobin levels on the first post-operative day, although fewer patients received RBC transfusion (tables 3-5).

Table 5: Post-operative Data[a]

Characteristics	CS	CS + Filter	Filter	Control	Effect of CS[b]	Effect of Filter[b]	p value[c]
12-h blood loss chest tube (mL)	728 ± 726	646 ± 487	772 ± 597	670 ± 444	0.90 (0.82-0.98)	1.02 (0.92-1.13)	0.04
Haemoglobin day 1 (mmol/L)	6.6 ± 0.9	6.6 ± 0.8	6.3 ± 0.8	6.1 ± 0.7	0.38 (0.26-0.49)	0.05 (-0.06-0.17)	0.15
Reexploration, n (%)	15 (8)	14 (8)	17 (10)	12 (7)	1.00 (0.56-1.80)	1.17 (0.65-2.11)	0.91
Myocardial infarction, n (%)	7 (4)	1 (1)	5 (3)	5 (3)	0.50 (0.14-1.73)	0.38 (0.11-1.32)	0.09
Stroke, n (%)	1 (1)	5 (3)	7 (4)	5 (3)	0.36 (0.10-1.22)	2.82 (0.82-9.62)	0.24
Ventilation time (h)	16.0 ± 23.9	14.9 ± 16.4	23.2 ± 43.5	21.3 ± 42.7	0.69 (0.55-0.86)	1.00 (0.80-1.26)	0.47
LOS intensive care unit (days)	1.9 ± 5.6	1.7 ± 2.4	2.4 ± 4.7	1.5 ± 1.7	0.95 (0.79-1.12)	1.18 (0.99-1.39)	<0.001
LOS hospital (days)	11.5 ±10.5	10.3 ± 7.8	12.7 ± 15.0	11.8 ± 9.6	0.89 (0.80-0.98)	0.98 (0.87–1.08)	0.08
One-year mortality, n (%)	1 (1)	6 (3)	8 (5)	5 (3)	0.36 (0.11–1.23)	3.32 (0.99–11.0)	0.21

[a] Data are presented as mean ± standard deviation, unless indicated otherwise. [b] Effects are ratio and 95% confidence intervals. A ratio larger than 1 indicates a higher risk in the control group. [c] The p value indicates the interaction effect of the CS in combination with the filter. CS, cell saver device; LOS, length of stay

Reexplorations were equally divided between the four groups (table 5). These 58 patients comprised 8% of the total study population, but consumed 30% of total RBC, 46% of total FFP, and 41% of total platelet concentrates. Exclusion of patients with a re-exploration did not change the result of the primary outcome.

Use of the CS resulted in a 31% (95% CI, 14% to 45%) shorter post-operative ventilation time. For the length of stay in the intensive care an interaction effect between CS and filter was significant. Without the CS, the use of a filter increased the length of stay by 60% (P<0.001). The length of hospital stay was reduced when the cell saver was used with a filter by 30% (P=0.002), but, when the filter was not used the cell saver increased the length of stay by 28% (p=0.048).

Discussion

This study demonstrates that during cardiac surgery, intra-operative use of a CS, with or without a filter, does not reduce the total number of allogeneic blood products that are transfused during hospital admission. However, the lower percentage of patients who received any transfusion when a CS was used is, from a clinical standpoint, equally important because transfusion with allogeneic blood products is associated with reduced long-term survival, increased morbidity and costs [12,13]. It is important to realize that this study had approximately 80% power to detect a 10% reduction in patients who required any allogeneic blood products and to detect an approximately 22% reduction in the number of blood products.

An explanation for these seemingly contradictory findings is that patients who were bleeding required more FFP's and platelets when a CS was used, which minimized the effect of the intervention in the overall population. The meta-analyses [3,4] did not show an association between CS use and FFP's, but the reported amounts of processed CS blood were small which may have obscured these effects. We processed all intra-operative wound blood, including cardiotomy suction and residual blood from the CPB circuit. This is reflected in the amount of retransfused blood, which was higher than in any of the published studies [2,5,14-17]. Unfortunately, we did not separately measure the blood collected before and after CPB and the cardiotomy blood. This would have given the opportunity to better characterize the effects of cell saving versus surgical bleeding on transfusion of haemostatic products.

Several differences with these previous investigations exist. We not only included coronary but also valve and combined procedures to reflect the usual clinical spectrum, which very few studies do [15]. Another difference is that this is a multicenter study

in which all centers used the same transfusion trigger. Cell-salvage procedures are usually tailored to local customs. This may explain why we found that the treatment effects were not consistent across the centers for all outcome measures. Another explanation is that protocol violations occurred. Violations were virtually absent for RBC transfusion (although clinical judgment was sometimes used to modify the transfusion threshold) but occurred in about 10% of FFP and platelet transfusions. In certain cases these violations were inevitable, for example in case of a large blood loss requiring immediate action, whereas in other cases intervention could wait until laboratory data were present.

We noticed also that a substantial amount of late RBC transfusions occurred, some of which fell outside the transfusion protocol. The majority occurred in patients with prolonged intensive care stay. Due to the blinding of the study, and hemoglobin levels between 4.5 (intensive care) and 5 mmol/L (ward) as transfusion trigger, a major effect on the results is unlikely. We did not exclude these cases from analysis as this reflects common clinical practice in the Netherlands. We believe therefore that our study provides a representative overall effect. This is supported by the fact that exclusion of the reexplorations, in which the highest number of platelets and FFP was used, did not change the results on the primary outcome.

We did not use point-of-care testing to guide transfusion decisions, as this was not state of the art when we conceived the study. Therefore, our transfusion protocol may have resulted in a too aggressive administration of FFP as the trigger was persistent blood loss combined with an increased prothrombin time (which most patients have already after CPB). This approach may not have produced the expected improvement in haemostasis. Point-of-care testing could better have guided transfusion of platelets or FFP as first haemostatic component, although already a substantial percentage of the platelets was transfused without concurrent administration of FFP.

Post-operative ventilation times were shorter when a CS was used. We also found higher post-operative haemoglobin levels, although fewer patients received RBC transfusion. Because of the washing process of a CS, approximately 1.4L of fluid was removed from the circulation in this study. Thus, a CS may act as haemoconcentrator by removing excess fluid and thus explain the shorter ventilation times.

There was no clinical relevant effect of the additional filtration of CS blood. Cell-saving device blood contains activated leukocytes with increased expression of integrins on both neutrophils and monocytes [18]. Furthermore fat and cytokines are incompletely removed by the washing process [19]. Leukocyte depletion filters can remove activated leukocytes microparticles and fat [6,8,19].

We included group 3 (filter) as part of the factorial study design to test the hypothesis that it is not mandatory to wash the blood with a CS when leukocyte depletion filters are extensively used. Kaza et al. [20] demonstrated that a 21-µm filter placed after the cardiotomy reservoir of the CPB circuit was able to remove fat micro-emboli completely. This approach was more effective than the use of a CS. Leukocyte depletion filters have an even finer mesh and also bind cells and particles through adhesion. Although the plasma fraction of the blood was preserved with this approach, transfusion requirements were higher and the post-operative ventilation time and length of stay in the intensive care and the hospital were longer. Using these filters alone cannot replace a CS and is not indicated as a routine technique in cardiac surgical patients.

In conclusion, this study shows that intra-operative use of a CS during cardiac surgery did not reduce the total number of allogeneic blood products, but its use reduced the percentage of patients who received allogeneic blood products. This finding has clinical implications, as transfusion of allogeneic blood products is associated with reduced long-term survival and increased morbidity. An additional filter did not result in a clinical relevant advantage. Finally, the novel approach to retransfuse all wound blood through a LD filter did not reduce allogeneic blood products and is not indicated in this setting. Our findings therefore support the routine use of a CS during cardiac surgery.

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Chapter 3

Do repeated runs of a cell saver device increase the pro-inflammatory properties of washed blood?

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Abstract

Background: Intra-operative cell salvage is increasingly used, especially in longer cases with continuing blood loss. However it is unknown if the quality of processed blood is affected when larger quantities of blood are processed. We hypothesized that the quality of the washed blood decreases after multiple runs.

Methods: Intra-operative cell salvage was performed in 42 consecutive patients undergoing cardiac surgery. When 1250 ml of blood was collected in the blood collection reservoir, this was processed and returned to the patient. In 21 patients more than 2500 ml of blood was collected during the whole procedure, thus allowing at least two subsequent runs with the auto-transfusion device. Blood samples were drawn from the blood collection reservoir of the cell saver device before, and from the processed blood after each run.

Results: After the first run interleukin-6 concentrations were reduced with 85% (from 21 ± 35 µg/l to 3.1 ± 4.4 µg/l), whereas after the second run 72% was removed (63 ± 69 µg/l to 17.6 ± 25.3 µg/l). Leukocyte counts almost doubled after both processing runs (from 2.6 ± 1.5 x 10^9/l to 5 ± 3.6 x10^9/l) and from 3.9 ± 2.2 x 10^9/l to 7.7 ± 5.9 x 10^9/l), haemoglobin concentration (14.8 ± 1.6 mmol/l versus 15.0 ± 1.1 mmol/l), free hemoglobin (2.3 ± 1.6 g/l versus 2.1 ± 1.4 g/l) and platelet counts (18 ± 9 x 10^9/l versus 28 ± 23 x 10^9/l) were not different between the two runs.

Conclusions: Our results suggest, based on interleukin-6 and free hemoglobin washout that the quality of the processed blood remains constant with multiple runs of the cell saver device.

Introduction

To minimize blood transfusion requirements during cardiac operations performed with cardiopulmonary bypass (CPB), the anti-coagulated shed wound blood can be salvaged with cardiotomy suction. However, there is pronounced inflammatory activity in cardiotomy suction blood [1], and retransfusion of cardiotomy suction blood has been shown to contribute to the post-operative inflammatory response [2-4].

Mechanical cell salvage devices are therefore increasingly used as an alternative method for intra-operative blood salvage. In a cell saver the shed wound blood is collected, washed and concentrated. The red blood cell concentrate is then retransfused in the patient.

Recently, the quality of this washed and concentrated blood was addressed in several studies [1,5,6]. It was found that processing of shed blood by a cell saver led to normalization of some, but not all, inflammatory markers in the processed blood. However, to our knowledge, these studies considered only one processing run of the cell saver. As a consequence it is unknown if the quality of processed cell saver blood is affected when larger quantities of blood are processed. We hypothesized that the quality of the washed blood decreases when, due to large blood loss, multiple runs of the cell saver are necessary. This has not been studied before and might have implications for the way cell savers are used during operations.

Therefore, in the present study we assessed the quality of the processed blood by measuring interleukin-6 (IL-6), leukocytes and free hemoglobin. After 1250 ml of wound blood was collected in the blood collection reservoir of the cell saver we measured the blood quality. After processing the collected blood we again measured the blood quality. We compared this to the quality of the collected and washed blood after a subsequent 1250 ml of wound blood was collected and processed.

Materials and methods

Patients

After written informed consent and approval by the institutional ethics committee on human research, intra-operative cell salvage was performed in 42 consecutive

patients presenting for elective coronary artery bypass surgery or first time aortic valve replacement. Excluded were patients with known coagulation disorders except for the use of aspirin or low molecular weight heparin given at least 10 h before surgery. We did not perform a power calculation, as this was a pilot study.

Clinical management

Anaesthesia was induced and maintained with propofol infusion, followed by 0.1 mg/kg pancuronium to facilitate intubation. Sufentanil (1-3 µg/kg) was administered in incremental doses. Before cannulation bovine lung heparin (300 IU/kg) was administered and supplemented as necessary to obtain activated clotting times (ACT, Hemochron, New Jersey, USA) in excess of 400 s. After cardiopulmonary bypass (CPB), heparin was neutralized by protamine in a 1:1 ratio. The CPB-circuit consisted of roller pumps (Stöckert, München, Germany) and a hollow fiber oxygenator (Cobe, Lakewood, CO, USA) primed with 500 ml hydroxyethyl starch 10% (Fresenius, Bad Homburg, Germany) and 1000 ml lactated Ringer's solution. Myocardial protection consisted of cold crystalloid solution (Plegisol, Abbott laboratories, IL, USA).

Cell salvage was achieved by using the CATS system (continuous auto transfusion system, Fresenius, Bad Homburg, Germany). The cell saver device was installed identically for every patient and every run was according to the manufacturer's instructions. Washing conditions for all patients and runs were set at the automated, quality wash program, incorporated in the machine by the manufacturer. The blood collection reservoir of the cell saver was primed with 100 ml of normal saline with 30.000 IU heparin/l added. All shed wound blood during the operation including blood from the operative field during CPB was collected in the cell saver reservoir. Cardiotomy suction was not used. Thus, all cardiotomy blood was also collected in the cell saver reservoir, thereby increasing the quantity of blood to be processed. The residual blood from the heart-lung machine after CPB was also processed by the cell saver, but this blood was not analysed in this study.

When 1250 ml of shed blood was collected in the blood collection reservoir of the cell saver this was processed and returned to the patient. If another 1250 ml of blood could be collected a second processing run was performed. The amount of 1250 ml was arbitrarily chosen, as this is a volume at which a clinically relevant transfusable amount

of blood can be acquired. Furthermore this amount reflects the actual amounts lost on average in CABG operations. It is also an economical balance between cost of an allogeneic blood transfusion and the disposable centrifuge unit.
Blood samples were drawn from the blood collection reservoir of the cell saver device before each run and from the processed and washed blood after each run.
The following parameters were measured: white blood cell (9 x 10^9/l) and platelet counts (9 x 10^9/l), hemoglobin (mmol/l), free hemoglobin (g/l), haematocrit (%), and the concentration of the pro-inflammatory cytokine interleukin-6 (IL-6, µg/l). Samples for cytokines were collected in ethylenadiamine tetraacetic acid (EDTA). The samples were centrifuged immediately and the resultant plasma was stored at -80°C until analysis. IL-6 was determined with a commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistics

Statistical analysis was performed with Wilcoxon signed ranks test as not all data had a normal distribution. A *p* value of less than 0.05 was considered significant. All the results are expressed as the mean ± standard deviation.

Results

In 21 of the 42 patients at least 2500 ml of blood was collected in the blood collection reservoir of the cell saver, allowing two runs with the auto-transfusion device. Fourteen of these patients underwent coronary artery bypass grafting and seven patients had aortic valve replacement. The total amount of salvaged blood was 4718 ± 1581 ml. This also included the residual heart-lung machine blood (911 ± 330 ml), but this blood was not analysed in this study. There were no allogeneic blood transfusions during the study period. The characteristics of the patients are summarized in table 1.

Table 1: Demographics

	Euro SCORE	Age (years)	Sex (m/f)	Height (cm)	Weight (kg)	CPB (min)
Study (n=21)	3.8 ± 2.2	66 ± 8.6	17/4	1.74 ± 0.0	82 ± 14	121.7 ± 29.7

Data are presented as mean ± SD; CPB, cardiopulmonary bypass; Euro SCORE, European System for Cardiac Operative Risk Evaluation

Processing of the blood resulted in a decrease of IL-6 in both runs. After the first run IL-6 levels were reduced with 85%, whereas after the second run 72% was removed. This difference was not significant. However the absolute reduction of Il-6 was larger in the second run. Leukocyte counts nearly doubled after each processing run (table 2).

Table 2: Results

Variable	Run 1: reservoir	Run 1: processed	Run 2: reservoir	Run 2: processed
IL-6 (µg/L) (n=18)	21 ± 35	3.1 ± 4.4[a]	63 ± 69[d]	17.6 ± 25.3[b,c]
Leukocytes (x10^9/L) (n=21)	2.6 ± 1.5	5 ± 3.6[a]	3.9 ± 2.2[d]	7.7 ± 5.9[b,c]
Free haemoglobin (g/L) (n=20)	2.2 ± 1.0	2.3 ± 1.64	2.3 ± 1.3	2.1 ± 1.4
Haemoglobin (mmol/L) (n=21)	2.7 ± 0.9	14.8 ± 1.6[a]	3.5 ± 0.9[d]	15.0 ± 1.1[b]
Haematocrit (n=21)	12 ± 0	71 ± 0[a]	16 ± 0[d]	72 ± 0[b]
Platelets (x10^9/L)(n=21)	72 ± 29	18 ± 9[a]	87 ± 32	28 ± 23[b]

Data are shown as mean ± SD

[a] p< 0.01 for reservoir versus processed 1; [b] p< 0.01 for reservoir 2 versus processed 2; [c] p<0.01 for processed 1 versus processed 2; [d] p<0.01 for reservoir 1 versus reservoir 2.

The concentrations of IL-6, leukocytes, hemoglobin and haematocrit significantly increased in the unprocessed blood during the course of the operation (table 2, reservoir 1 vs. reservoir. The concentration of free hemoglobin in the blood collection reservoir of the cell saver did not increase in the unprocessed blood (table 2, reservoir 1 vs reservoir 2). Processing of blood from the cell saver blood collection reservoir resulted, in both runs, in a significant increase in hemoglobin, haematocrit and a significant decrease of platelets. The hemoglobin concentration, free hemoglobin concentration and platelet counts were not different between the two processed runs (table 2).

Discussion

The results of the present study demonstrate that repeated processing runs of shed wound blood with a cell saver leads to a similar reduction of the concentration of the pro-inflammatory cytokine IL-6. Leukocytes are retained. Hemoglobin, free hemoglobin, haematocrit and platelet concentrations were also not different between

the two processed runs. Thus, we showed that subsequent runs with a cell saver do not diminish the washout ability of pro-inflammatory markers.

Our IL-6 concentrations in the salvaged wound blood were relatively low compared to other studies that investigated the IL-6 concentrations in salvaged wound blood. An explanation may be that in this study only salvaged wound blood was used whereas in other studies the residual heart-lung machine blood was also processed [5,6]. As in earlier studies we found a high standard deviation of IL-6 values between and in patients [1]. We did not measure the IL-6 concentration in the patients, as in this pilot study we were only interested in the blood quality aspect of the cell saver. The significant increase in IL-6 in the unprocessed blood between the two runs is most likely due to the ongoing inflammatory response during the course of the operation and CPB [3,7]. Hemodilution by priming of the cell saver reservoir is a less likely explanation for the lower IL-6 concentrations in the first blood collection reservoir. The priming volume was 100 ml and would thus have a dilution effect of 8%.

Free hemoglobin levels were not reduced. Approximately 0.5% of erythrocytes are being damaged during and after the washing procedure, resulting in new hemoglobin release [8].

Our study also demonstrates a concentrating effect of leukocytes after each run. This has been demonstrated previously [1,9] and might be explained by the centrifugation process itself. White blood cells, which are larger but not heavier than red blood cells, are spun to the rim of the washing chamber, the so-called buffy coat. With on-going centrifugation and washing the buffy coat becomes so concentrated with debris that leukocytes pass with the red blood cells into the process bag. The formation of such a layer of debris has been demonstrated before [9,10].

The consistent performance of red blood cell saving can be explained by the operating process of the cell saver itself. A light sensor reacts on the red blood cell concentration and starts the pump to transport the processed blood to the collection bag. Thus one would, given time, always expect similar hemoglobin and haematocrit in subsequent runs.

The device used in this study is the CATS cell saver. This is a continuous auto-transfusion system, which means that the system runs continuously until the blood collection reservoir is empty. This is in contrast to a bowl system, which operates by

using batches of blood from the blood collection reservoir. In the current study we used this continuous system as a bowl collection system for a clear separation of the amount of blood processed.

In retrospect in a few patients it would have been possible to perform a third processing run. It would have been interesting to see the effect of a further processing run on the blood quality. Furthermore, measurement of leukocyte activation in the processed blood could have shown if leukocytes are activated by the blood processing process, giving further information on processed blood quality. In conclusion, our results suggest that with repeated runs blood quality is maintained. With repeated runs of the cell saver device leukocytes are retained. This is probably due to a concentration effect.

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Chapter 4

Additional post-operative cell salvage of shed mediastinal blood in cardiac surgery does not reduce allogeneic blood transfusions: a cohort study

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Abstract

Background: Does additional post-operative collection and processing of mediastinal shed blood with a cell salvage device reduce the number of allogeneic blood transfusions compared to intra-operative cell salvage alone.

Methods: Single centre cohort study in which 99 adult patients with coronary artery bypass grafting or aortic valve replacement were allocated to either a C.A.T.S® group with intra-operative blood processing only or a CardioPat® group with both intra- and post-operative blood processing. The primary endpoint was the number of allogeneic blood transfusions during hospital admission.

Results: The study included 99 patients, 50 in the C.A.T.S® and 49 in the CardioPat® group. There was no difference between groups in the number of RBC (C.AT.S® group 43 units versus CardioPat® 50 units, p= 0.74), number of FFP (C.A.T.S® 8 units versus CardioPat® 8 units, p=1.00) or platelets (C.A.T.S® 5 units versus CardioPat® 4 units, p=1.00) transfused during hospital stay. Creatinine kinase (CK) levels were not different between groups three hours after arrival in the ICU (CardioPat® group versus CATS® group, p= 0.17)). But compared to the CATS® group on the first (CK 416 IU/L ± 355 IU/L) and second post-operative day (CK 418 IU/L ± 380 IU/L) the increase in CK levels was more in the CardioPat group on the first (CK 640 IU/L ± 668 IU/L, p=0.02) and second post-operative day (CK 658 IU/L ± 723 IU/L, p=0.05). There was no difference over time in the levels of Troponin T (p=0.67) or CK-MB (p=0.43).

Conclusions: Post-operative cell salvage does not reduce transfusion requirements compared to intra-operative cell salvage alone but results in elevated total CK levels that indicates haemolysis.

Introduction

Intra-operative salvage of shed autologous blood with a mechanical device (cell saver) is a well-established blood conservation strategy to reduce allogeneic blood transfusion during cardiac surgery [1]. Blood conservation strategies are also expanded beyond the intra-operative period. Post-operative auto transfusion of unwashed shed mediastinal blood (SMB) has been studied before, but was largely abandoned for fear of inducing coagulopathy with the retransfusion of the activated and inflammatory blood and is not recommended by the current guidelines [2]. However, recent studies suggest that there is no impairment of haemostasis or an increase in blood loss with the retransfusion of unwashed SMB and that this procedure is an effective way to reduce allogeneic blood transfusions [3, 4], although SMB contains a substantial amount of potential embolic substances, activated platelets and pro-inflammatory substances [5]. Several studies have been undertaken to investigate the (additional) efficacy and safety of washing and processing post-operative SMB with a cell saver device before retransfusion [6-10]. Current guidelines now advocate this method, but only with a class III recommendation [2].

Recently a new type of cell saver (CardioPat®, Haemonetics) has become available. By its novel design, using a small collapsible processing disk and well-regulated level of suction, this cell saver is particularly suitable for continuous intra-operative and post-operative use [10].

This cohort study investigates whether additional post-operative collection and processing of mediastinal shed blood with a cell salvage device that was also used intra-operatively would reduce the number of allogeneic blood transfusions in patients undergoing cardiac surgery compared to intra-operative cell salvage alone.

Methods

This retrospective single centre cohort study comprised 99 adult patients scheduled for either non-emergent coronary artery bypass grafting (CABG) or first time aortic valve replacement (AVR). Excluded were patients with off-pump CABG, patients with known coagulation disorders except for the use of aspirin, and patients with

pre-existing liver disease or renal dysfunction. The institutional review board of the University Medical Centre Groningen approved the study. Patients gave signed informed consent.

Fifty patients were operated using the continuous auto-transfusion system (C.A.T.S®, Fresenius, Bad Homburg, Germany) for intra-operative blood processing. To minimize time effects, these patients were the last 50 patients from a previous study (ISRCTN 58333401), which studied the effects of cell saver, leukocyte depletion filters and their combination [11]. 49 consecutive patients were operated using the CardioPat® (Haemonetics, Braintree, USA) as part of a clinical evaluation for this device in our hospital. The study period was from 2009 to 2010. Patients were followed up until hospital discharge, without loss to follow up.

All patients received standard anaesthesia, consisting of propofol, and sufentanil (1-3 µg/kg). Ventilation was performed with an inspiratory oxygen fraction of 0.4, a tidal volume of 6-8ml/kg and a respiratory rate adjusted to maintain normocapnia. Protease inhibitors were not used. The CPB circuit consisted of roller pumps and an open venous reservoir and was primed with 1000 ml lactated Ringer's solution and 500 mL hydroxyethylstarch 10% (Fresenius, Bad Homburg Germany). Pump flow was set at 2.4 L/m^2/min and temperature was allowed to drift to 34°C. Anticoagulation was performed with heparin (3 mg/kg) and additional doses if required to maintain an activated clotting time (ACT), greater than 400 sec. Cardioplegia was performed with either blood or crystalloid solution.

In both groups, all blood suctioned from the wound and pleural space from incision until wound closure was processed with the cell saver and retransfused. Thus, conventional cardiotomy suction was not used during CPB. Residual blood in the heart lung machine circuit after the end of CPB was also processed with the cell saver and retransfused into the patient. Both cell savers were set-up according to the manufacturers instructions. The reservoir of the cell saver was primed with 100 ml of normal saline with 30.000 IU/L of heparin. During the operation there was a continuous flush of heparinised saline through the cell salvage suction tube. In the CATS® group the cell salvage was not used in the post-operative period. In the CardioPat® group however, cell salvage was continued during the first 6 post-operative hours. During that time all SMB was collected, processed and returned to the patient. After the first 6 hours

the shed blood was no longer processed or returned. This is in accordance with the manufacturer's instructions and the guidelines of the American Association of Blood Banks [12]. In contrast to the intra-operative period, there was no anticoagulation added to the reservoir of the CardioPat® in the post-operative period as per manufacturer's instructions.

The transfusion protocol prescribed that RBC's were to be transfused when the post-operative haemoglobin level was <8 g/dl. FFP was transfused in case of excessive bleeding (>150 ml/h for 2 consecutive hours and International Normalized Ratio (INR) or Prothrombin Time (PT) >1.5 normal). Platelets were transfused when platelet counts were $<100x10^9$/l in combination with excessive bleeding. The decision for surgical re-exploration was made on the usual clinical grounds.

The primary endpoint of this study was the number of allogeneic blood transfusions during hospital admission. Secondary endpoints were the percentage of patients that received allogeneic blood products, amount of post-operative blood loss (defined as the total amount of blood loss from closure of the sternum until 12 hours post-operatively), myocardial damage and renal dysfunction, number of re-explorations, length of stay in the ICU and hospital, the number of post-operative complications (myocardial infarction, atrial fibrillations and stroke). Myocardial damage was assessed by EKG changes and routine enzymatic measurements (creatine kinase (CK), myocardial band (MB) isoenzymes of CK and troponin T). Renal function was assessed by measuring serum creatinine. Routine coagulation test (PT, APTT and fibrinogen) were used to assess the coagulation profile. Blood samples were taken pre-operatively, after end of CPB, after admission in the ICU, the morning of the first post-operative day and the morning of the second post-operative day and at hospital discharge.

Statistical analysis

Continuous data were analysed using Student's t test or the Mann-Whitney U-test as appropriate. Blood transfusion data were analysed using Poisson regression for count variables and logistic regression for binary variables. Categorical variables were analysed using the chi-square test or Fisher's exact test as appropriate. To achieve

an approximately normal distribution of the biochemical markers we applied log conversion. We then used repeated measurements analysis of variance for serial data. A p value of < 0,05 was considered statistically significant. We calculated the sample size for this study as follows. Preliminary data based on the first results of our trial (ISRCTN 58333401) showed a mean transfusion rate of two units RBC and a standard deviation of two units. Mean post-operative chest tube lost was about 700 ml. This corresponded to a processed volume of at least 1 unit of RBC. Therefore to reach a reduction by 33% in RBC about 50 patients would be necessary in each group with the usual assumptions of an alpha 0.05 and a beta 0.8.

Results

The study included 99 patients, 50 in the CATS® group, and 49 in the CardioPat® group. Data of the post-operative blood collection of one patient in the CardioPat® group was missing; all other data from this patient were used.
Patient demographics revealed no differences between the groups (table 1).

The residual volume of CPB blood was higher in the CATS® group, whereas more intra-operative blood was collected in the CardioPat® group (table 1). Post-operative blood loss in the first 12 hours was 482 ± 339 mL in the CATS® group and 654 ± 523 mL in the CardioPat® group (p=0.55). From this blood, 474±363 mL was processed with the CardioPat® after 6 hours which resulted in 141 ± 122 mL of blood that was retransfused.The haemoglobin level of the processed blood from the CardioPat® was 25.3 ± 3.0 g/dL corresponding to a haematocrit of 70 ± 5 % (n=12). However, the extraction ratio (i.e. the amount of processed blood divided by the amount of collected blood) of the CardioPat® was lower than the extraction ratio of the CATS® (table 1).
Table 2 shows the transfusion data with confidence intervals. There were no differences between the groups in the number of units of red blood cells (RBC) that were transfused or in the number of patients who received RBC transfusions (table 2).

Table 1: Patient demographics and intra-operative data

	CATS® (n=50)	CardioPat® (n=49)	p-value
Age (years)	66.8 ± 9.7	65.5 ± 9.3	0.48
Sex (m/f)	36/14	42/7	0.09
Euro SCORE	4.8 ± 3.2	4.5 ± 3.0	0.67
CABG/AVR (n)	37/13	38/11	0.68
Previous myocardial infarction (n (%))	12 (24%)	7 (14%)	0.22
Pulmonary disease (n (%))	5 (10%)	7 (14%)	0.51
Hypertension (n (%))	24 (48%)	26 (54%)	0.61
Diabetes (n (%))	12 (24%)	13 (27%)	0.77
Previous Cerebrovascular accident (n (%))	0 (0%)	1 (2%)	0.31
Aspirin (n (%))	28 (56%)	35 (72%)	0.11
Haemoglobin (g/dL)	12.2 ± 1.3	12.3 ± 1.3	0.35
Creatinine (umol/L)	76 ± 15	82 ± 24	0.15
Aortic clamp time (min)	62 ± 27	63 ± 20	0.94
Perfusion time (min)	104 ± 41	102 ± 27	0.75
Haemoglobin at end of operation (g/dL)	8.2 ± 0.8	8.3 ± 1.1	0.30
Residual volume CPB (mL)	922 ± 352	752 ± 149	< 0.01
Intra-operative collected blood (mL)	1929 ± 766	2470 ± 895	< 0.01
Processed blood (mL)	605 ± 247	600 ± 207	0.9
Extraction ratio cell saver device	0.32 ± 0.09	0.25 ± 0.05	< 0.01

Results are presented as number of patients (n) and percentage (%) or mean ± standard deviation (SD), as indicated. Euro Score, European System for Cardiac Operative Risk Evaluation; CABG, coronary artery bypass grafting; AVR, aortic valve replacement; CPB, cardiopulmonary bypass

Table 2: Transfusion Data

	CATS® (n=50)	CardioPat® (n=49)	p value	Odds Ratio [95% confidence interval]
Units RBC transfused first 24 hrs (n)	31	26	0.80	0.99 [0.87-1.12]
Patients transfused first 24 hrs (n (%))	11 (22%)	11 (22%)	0.96	1.02 [0.39-2.64]
Units RBC transfused during hospital stay (n)	43	50	0.74	1.00 [0.80-1.24]
Patients transfused (n (%))	14 (28%)	12 (25%)	0.69	0.83 [0.34-1.04]
Units FFP transfused (n)	8	8	1.00	1.00 [0.80-1.24]
Patients transfused FFP (n (%))	2 (4%)	3 (6%)	0.68	1.56 [0.25-9.80]
Units platelets transfused (n)	5	4	1.00	0.97 [0.58-1.68]
Patients transfused platelets (n (%))	4 (8%)	4 (8%)	1.00	1.02 [0.24-4.33]
Total allogeneic transfusion (n)	56	62	0.61	1.00 [0.94-1.06]

Results are presented as number of patients (n) and percentage (%) or mean ± standard deviation (SD), as indicated.

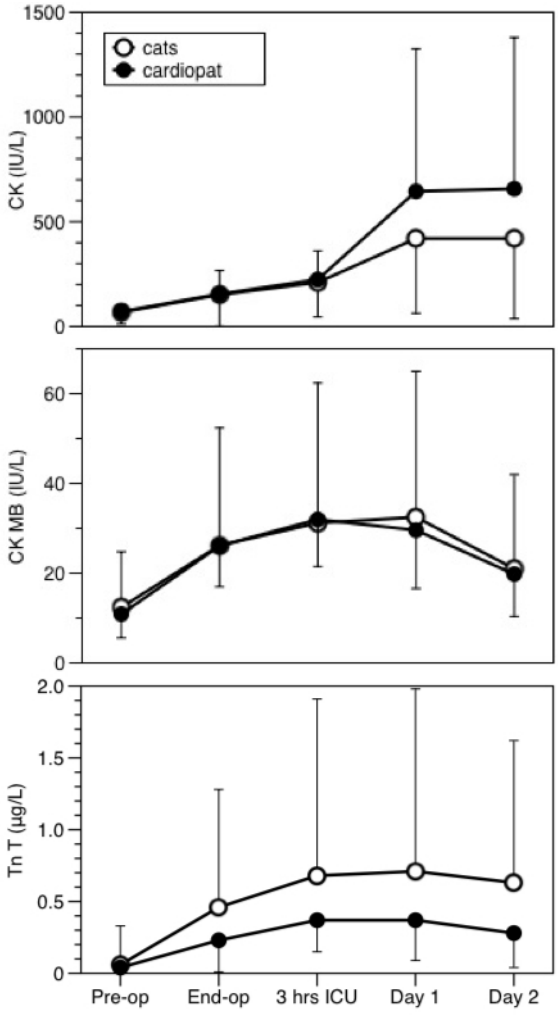
The transfusion load of the RBC's in the first 24 hours therefore equalled 0.6 U per patient in the CATS® group and 0.5 U per patient in the CardioPat® group (p=0.74). This amounted to 2.8 U per transfused patient in the CATS® group and 2.4 U per transfused patient in the CardioPat® group (p= 0.54). There was also no difference between the groups in the number of units of FFP or platelets that were transfused (table 2). There was no difference in post-operative blood loss between cell savers, although there was a trend towards more blood loss in the CardioPat® group.
In table 3 the results of the routine coagulation tests are shown. There was no clinical relevant difference between the groups.

Table 3: Coagulation test

	Time point	CATS® (n=50)	CardioPat® (n=49)	p-value
Prothrombin Time (sec)	Pre-operative	11.6 ± 1.5	11.0 ± 0.5	0.01
	ICU	13.4 ± 2.5	12.6 ± 1.0	0.03
	Day1	11.7 ± 1.5	11.3 ± 0.7	0.04
Activated Partial Thromboplastin Time (sec)	Pre-operative	30.9 ± 24.9	26.6 ± 2.2	0.24
	ICU	32.7 ± 5.9	30.2 ± 3.9	0.02
	Day1	26.8 ± 2.6	27.4 ± 2.6	0.25
Fibrogen (g/l)	Pre-operative	3.2 ± 0.7	3.4 ± 0.8	0.31
	ICU	2.0 ± 0.6	1.9 ± 0.5	0.32
	Day1	2.8 ± 0.8	3.0 ± 0.9	0.23

Creatinine kinase levels were not different between groups 3 hours after arrival in the ICU (CardioPat® group (226 IU/L ± 135 IU/L) and CATS® group (210 IU/L ± 165 IU/L, p= 0.17)), but increased more in the CardioPat® group on the first (640 IU/L ± 668 IU/L) and second post-operative day (658 IU/L ± 723 IU/L) compared to the CATS® group on the first (416 IU/L ± 355 IU/L, p=0.02) and second post-operative day (418 IU/L ± 380 IU/L, p=0.05) figure 1). There was no difference between the groups at the time points in the levels of Troponin T (p=0.67) or CK-MB (p=0.43, figure 1).

Figure 1: Perioperative course of biochemical markers



Results are presented as mean ± standard deviation (SD). Pre-op, before operation; end-op, end of operation; ICU, intensive care unit; Day 1 and 2, post-operative day 1 and 2; CK, Creatine kinase; CK MB: Creatine kinase myocardial band; TnT, Troponin T.

There were no differences in the post-operative data as shown in table 4.

Table 4: Post-operative data

	CATS® (n=50)	CardioPat® (n=49)	p value
Post-operative collected blood over 12hrs (mL)	482 ± 339	654 ± 523	0.55
Post-operative processed blood (mL)	Na	474 ± 363	Na
Retransfused post-operative blood volume (mL)	Na	141 ± 122	Na
Extraction ratio	Na	0.29 ± 0.11	Na
Re-explorations (n (%))	2 (4%)	2 (4%)	1.00
Myocardial infarction (n (%))	1 (2%)	0 (0%)	1.00
New atrial fibrillation (n (%))	12 (24%)	10 (21%)	0.81
Stroke (n (%))	0 (0%)	0 (0%)	1.00
Length of stay intensive care (days)	1.2 ± 1.0	1.3 ± 1.4	0.76
Length of stay hospital (days)	9.3 ± 4.7	10.8 ± 16.2	0.70

Results are presented as patients (n)/percentage (%) or mean ± standard deviation (SD), as indicated.
NA, not applicable

Discussion

We found that additional post-operative salvage and washing of the shed mediastinal blood after intra-operative cell salvage neither reduced the number of allogeneic RBC transfusions nor the number of patients transfused, compared to intra-operative cell salvage alone. Autotransfusion of post-operative SMB processed with a cell saver, which was also used intra-operatively, has been studied before [7, 8, 10, 13]. In one study a reduction in the number of RBC's transfusions, but not in the number of patients transfused was found [7]. A reduction in the number of RBC's used and in the number of patients transfused was reported in another study, but in this study the intra-operative blood loss during CPB or residual CPB blood was not processed with the cell saver [8]. However, in both studies the control group did not have intra-operative cell salvage at all. Therefore these studies provide no information on the additional effect of post-operative cell salvage. In another study where cell salvage was used only for the post-operative period the number of patients that was transfused with RBC's was significantly reduced [9].

One recent study used a similar cell saver deployment as we did [10]. In contrast to our results, a significant reduction in the mean transfusion requirements of RBC's was found. For an explanation of these contradictory results it is necessary to know the blood volumes that are collected and processed by the cell saver in order tot assess the efficacy of the blood salvage procedure. Unfortunately, most of the mentioned studies do not report these volumes. Weltert et al [10] only report the amount of returned processed blood after 6 hours (350 ml) and the total amount of 24-hour blood loss (720 ml). Considering a typical extraction ratio for blood processing in the order of 0.3 (as we found for both devices), this means that to retransfuse a mean processed amount of blood of 350 ml the original input must have been at least 1000ml in 6 hours, i.e. much more than the fore mentioned amount of total 24-hour blood loss. These volumes are rather high and have a significant impact on allogeneic transfusion data, as around 200-250 mL of processed cell saver blood equals one unit of packed cells. This may therefore explain the different results.

Since the introduction of the transfusion of post-operative unwashed SMB it is known that markers of myocardial damage rise [14]. In our study, the patients who received post-operatively washed SMB had a significant higher level of total CK compared to those that did not receive washed SMB whereas the other markers of myocardial damage such as CK-MB and troponin T were not elevated. The rise in total CK levels was therefore not of cardiac origin. The rise in total CK started after processed blood was retransfused to the patient in the intensive care unit and continued up to 48 hours post-operatively. Conventional anticoagulation with heparin was used in both groups during the intra-operative part of the blood salvage. However in the post-operative setting the CardioPat® does not require anticoagulation of the blood in the reservoir. This likely induces extensive haemolysis in the reservoir and therefore a rise in CK levels that persists despite the washing of the blood.

Compared to earlier studies on auto transfusion of unwashed SMB the rise in creatine kinase was much lower [14-16]. This might be attributed to the washing of the post-operative shed and highly inflammatory mediastinal blood itself, further indicating that the washing process per se improves blood quality [17, 18]. Nonetheless, our data suggest that caution should be exerted even when washed SMB is retransfused.

There are several limitations to our study. First the study was observational and small compared to other studies with post and intra-operative cell saver use. However, clinical practice was strict and the patient demographics demonstrated similar groups. Given the sample size calculation and the confidence limits of the main results it is unlikely that this affected our results. Moreover the quantity of SMB in our study was comparable to the reported quantities of SMB in other studies. A second limitation is that we did not measure the concentration of CK's of the SMB before being processed. In conclusion, continuing cell salvage beyond the operating room does not reduce transfusion requirements compared to intra-operative cell salvage alone but results in elevated total CK levels that suggest haemolysis.

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Chapter 5

Clinical efficacy and biocompatibility of three different leukocyte and fat removal filters during cardiac surgery

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Abstract

Background: Activated leukocytes and fat particles are associated with organ injury after a cardiac surgery. Filters are currently used to remove either leukocytes or fat particles. A novel approach with a filter that combines leukocyte and fat removal might be clinically useful. As it is not known which type of filter has a good and safe performance in both leukocyte and fat removal, we measured in this study the leukocyte and fat removal properties and the biocompatibility of three different filters.

Methods: We used six Pall RS1 (Pall, Portsmouth, England) leukocyte removal filters, six Pall LipiGuard fat removal filters, and six Fresenius Biofil 02 (Fresenius, Emmer-Compascuum, The Netherlands) leukocyte removal filters and measured the passage times of 500 and 1000 mL of residual heart–lung machine blood. We determined the circulating leukocyte and platelet counts, and total haemoglobin, triglyceride, and free fatty acid concentration after the filters. In addition, we measured free haemoglobin, plasma elastase (Merck, Darmstadt, Germany), and complement C5–9 (Quidel, San Diego, CA, U.S.A.) to assess the biocompatibility of the filters. The circulating fat particles were calculated with an automated haematology analyzer.

Results: The passage time for the blood was shortest for the Biofil filter ($P = 0.02$, analysis of variance). The total leukocyte counts ($P = 0.04$) and fat particles ($P = 0.02$) were higher after the LipiGuard filter. This filter also had a higher increase in free haemoglobin concentration ($P = 0.03$).

Conclusions: We conclude that the leukocyte removal filters were superior to the fat removal filter both in leukocyte and fat removal.

Introduction

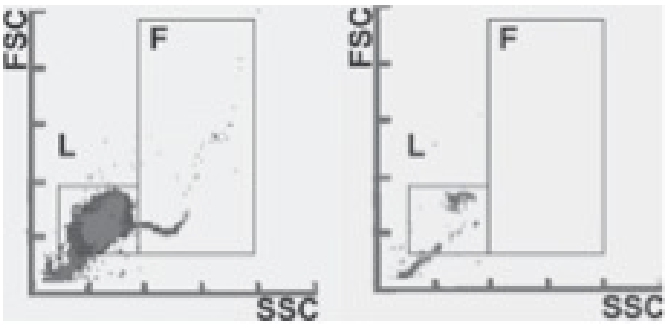
Activated leukocytes and fat particles are associated with organ injury in patients after a cardiac surgery. The effects on the lungs, brain, and kidneys have been documented [1-3]. Leukocyte depletion by means of filtration has been proposed as a method to reduce this organ injury [4,5]. Recently, it was suggested to remove, in addition, fat particles from the circulation by filtration [6]. Filters may therefore be used to remove either leukocytes or fat particles during a cardiac surgery. However, by its structure and nature a leukocyte removal filter also removes fat particles [7], and a fat removal filter also removes leukocytes, be it not at the same efficiency [8]. A novel approach with a filter that combines both properties might be clinically useful. However, we do not know which type of filter has a good performance in a clinical setting during a cardiac surgery in both leukocyte and fat removal, while at the same time the blood damage and blood activation are minimal. Most clinical filtration procedures are currently performed during a cardiopulmonary bypass (CPB) with its concomitant haemodilution. Haemodilution may increase the volume capacity of the filters, but to what extent is unknown. A large filter capacity would improve the clinical acceptability as it minimizes filter changes.

Therefore, we measured in this study the leukocyte and fat removal properties and the biocompatibility of three different filters using residual heart–lung machine blood.

Methods

After a local ethics committee approval and patient consent, we collected in this prospective randomized study the residual blood that was left in the heart–lung machine after a CPB of 18 consecutive cardiac surgical patients. The patients underwent either a coronary artery bypass grafting and/or a valve replacement. This blood was filtered before re-transfusion in a patient with a leukocyte or a fat removal filter under gravity from a height of 140 cm. This height equals a pressure of 100 mm Hg. We used one filter for each patient. As filters, we used six Pall RS1 (Pall, Portsmouth, England) leukocyte removal filters, six Pall LipiGuard fat removal filters, and six Fresenius Biofil 02 leukocyte removal filters (Fresenius, Emmer-Compascuum, The Netherlands). We

measured the time of the passage of 500 and 1000 mL of the blood. We also took three blood samples: one sample from the transfusion bag with the residual blood, one sample after the filter at 500 mL, and one sample after the filter at 1000 mL. From these blood samples, we determined the circulating leukocyte and platelet counts, and the total haemoglobin, triglyceride, and free fatty acid concentration. In addition, two blood samples were collected in ethylenediaminetetraacetic acid medium: one sample from the residual blood bag and one sample after the filter at 1000 mL, or less when the filter was blocked. These samples were immediately centrifuged at 1000 ×g for 10 min. The plasma was collected and stored at -80°C until further analysis. From these samples, we measured free haemoglobin as a measure of erythrocyte lysis, the elastase (Merck, Darmstadt, Germany) concentration as a measure of leukocyte activation, and the complement C5–9 (Quidel, San Diego, CA, U.S.A.) complex concentration as a measure for complement system activation using an enzyme immunoassay. These three measurements served to estimate the biocompatibility of the filters.



A B

Figure. 1. Two plots from an automated haematology analyzer taken from the residual heart–lung machine blood (A) and from blood that has passed the filter (B). The plots are constructed using front-scattered (FSC) and side-scattered (SSC) laser light. Leukocytes are in the rectangular L; fat particles form a sigmoid- shaped curve in the rectangular F. In panel B, both leukocytes (L) and fat particles (F) are reduced.

The circulating fat particles were calculated with an automated haematology analyzer based on fluorescence flow cytometry (Sysmex XE-2100; Sysmex, Kobe, Japan) as previously described [9]. Briefly, cells are counted in the first channel of the analyzer using front- and side-scattered (SSC) laser light. Fat particles also diffract the laser light resulting in a sigmoid-shaped curve in this channel (Fig. 1A).

The fat particles are thus also counted. The cells are differentiated and again counted in the second channel using fluorescence and SSC laser light. The cell counts from the second channel are subtracted from the total counts from the first channel. The difference reflects the circulating fat content. Statistical analysis was performed using Student's t-test for paired values and analysis of variance (ANOVA) with Bonferroni post hoc analysis as appropriate. Two-way ANOVA for repeated measurements was used to determine the effects of quantity, group, and interaction over the three measurement points. $P < 0.05$ was considered significant. Values are given as mean ± standard deviation. For the fat and particle counts, values are given as a percentage of the individual patient's starting value.

Result

The patient demographics are shown in Table 1, and indicate that the groups were not different. The volume of the residual blood was 1197 ±323 mL and was not statistically different between the groups.

Tabel 1: Demographics

	RS 1 (n=6)	LipiGuard (n=6)	Biofil 02 (n=6)	
Age (year)	65 ± 9	61 ± 11	70 ± 7	NS
Height (cm)	176 ± 5	174 ± 15	175 ± 6	NS
Weight (kg)	79 ± 7	85 ± 7	87 ± 17	NS
Male	6	5	6	NS
CABG	3	4	3	NS
Valve	2	2	1	NS
CABG + valve	1	0	2	NS
CPB time (min)	133 ± 73	121 ± 38	102 ± 26	NS

CABG, coronary artery bypass graft; CPB, cardiopulmonary bypass; NS, not significant.

In three patients, the total amount of residual heart–lung machine blood was <1000 mL (550 and 950 mL RS 1 group, and 850 mL LipiGuard group). In these patients, the third (1000 mL) sample was collected at these blood quantities. Three of the LipiGuard filters became blocked before 1000 mL had passed (at 500, 600, and 650 mL, respectively). In these patients, the third (1000 mL) sample was collected at these blood quantities and the passage time was set at 600 s. The total passage time for the blood was shortest for the Biofil filter ($P = 0.02$, Fig. 2).

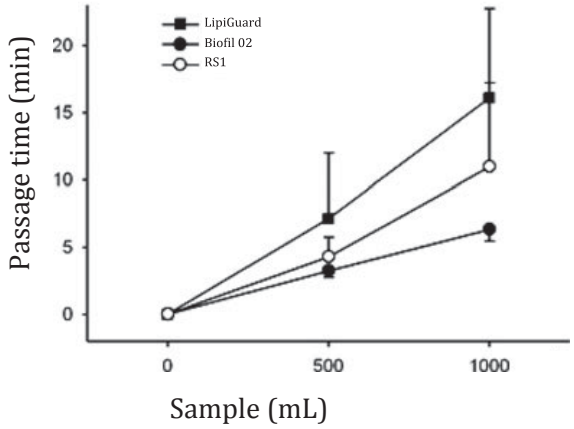


Figure. 2. Passage time for residual heart–lung machine blood through three types of leukocyte and fat removal filters. Error bars indicate standard deviation. Repeated measurement analysis indicates a difference (P = 0.03) between the filter types.

This was not only true for the first 500 mL of the blood, but especially for the second 500 mL. All filters removed leukocytes, but the total leukocyte counts were significantly higher after the LipiGuard filter ($P = 0.04$) at 500 and 1000 mL (Table 2). The leukocyte counts after the second 500 mL were slightly higher in all filters than after the first 500 mL (Table 2). The circulating platelet counts were lowest after the RS1 filter ($P = 0.05$, Table 2). The concentration of free fatty acids in the blood after passage through the three filter types was almost similar. However, fat particles were less reduced in the LipiGuard filter at 500 mL ($P = 0.02$). A typical example of these fat particles in the blood before filtration and after 500 mL of the blood had passed through the filter is shown in Fig. 1.

Table 2: Composition of the residual heart–lung machine blood before and after filtration with three different filters

Filter	RS 1			LipiGuard			Biofill		
Sample point (mL)	0	500	1000	0	500	1000	0	500	1000
n	6	6	5	6	6	4	6	6	6
Haemoglobin (mmol/L)	4.6±0.6	-	-	4.4±1.1	-		4.2±1	-	-
Leukocytes (x10^9/L)	8.3±2.6	0.13±0.05‡	0.26±0.18‡	7.3±3.2	3.0±2.3‡‡	4.1±2.5‡‡	7.8±4.2	0.13±0.05‡	0.22±0.12‡
Platelets (x10^9/L)	129±32	4.8±2.9*‡	5.6±1.8*‡	151±40	64±38‡	87±118‡	154±66	56±72‡	83±17
Triglycerides (mmol/L)	0.41±0.07	0.37±0.06	0.37±0.06	0.54±0.23	0.53±0.23	0.48±0.13	0.53±0.17	0.51±0.15	0.51±0.16
Free fatty acids (µmol/L)	1011±445	852±391	797±410‡	943±260	881±266	815±194	961±222	865±171‡	868±174
Elastase (µg/L)	822±179	-	1167±102‡	929±411	-	1357±338‡	820±282	-	1044±264‡
C5-9 (ηg/L)	1454±756	-	1877±771‡	1874±516	-	1994±509	1568±968	-	1854±889
Plasma hemoglobin (mg/L)	356±175	-	388±156	305±54	-	1247±973‡‡	185±123	-	207±143
Fat particle decrease (%)	-	86±8	86±8	-	50±22‡	38±17‡	-	83±9	92±8

*P ≤ 0.05 for RS 1 versus LipiGuard and Biofil; ‡P ≤ 0.05 for LipiGuard versus RS 1 and Biofil; ‡P≤ 0.05 to residual blood.

The increase in the elastase concentrations was slightly lower after the Biofil filter ($P = 0.26$, Table 2). The increase in the complement C5–9 concentration was slightly, but not significantly, lower in the LipiGuard filter ($P = 0.21$, Table 2). This filter also showed a significant larger increase in plasma haemoglobin concentration than the other filters ($P = 0.03$, Table 2). There was no correlation between the elastase concentration or the complement C5–9 concentration in the residual blood and the duration of CPB or the quantity of residual blood. A small but significant negative correlation was observed between the quantity of the residual blood and the plasma haemoglobin concentration ($R = -0.51, P = 0$).

Discussion

This study shows a marked difference in passage time, in leukocyte and platelet removal, and in fat removal between the three filter types. Moreover, the biochemical measurements also demonstrate a difference between the three filter types in the increase in elastase, complement C5, and free haemoglobin after the passage of 1000 mL of residual heart–lung machine blood. The passage times for the residual heart–lung machine blood were different between the three groups. Both leukocyte depletion filters performed better than the fat removal filter. As these filters will be used clinically in the operating theater, a high flow rate and a large capacity are important. We used a constant pressure on the filter, and thus, the flow rate is reflected in the passage time. All filters showed an increase in the passage time for the second 500 mL of the blood. This suggests that the filter gradually becomes saturated with cells and particles. The capacity of the filters may therefore be estimated by the increase in the passage time for the second 500 mL and by the increase in the leukocyte counts. A small increase in passage time for the second 500 mL of the blood and in leukocyte counts suggests that 1000 mL of the residual heart–lung machine blood, with a low haemoglobin concentration, may be safely processed by the leukocyte filters. Unfortunately, our study cannot answer the question if, apart from debris and particles, the filters become blocked by the passage of the red blood cells or the leukocytes. The leukocyte load of the residual heart–lung machine blood was not particularly high compared to normal pre-operative patient values, but the leukocytes

have been activated by the CPB circuit. We have previously shown that activated leukocytes are preferentially trapped in the filter [10], and thus may lead to a rapid saturation of the filter. We did not differentiate the leukocyte counts into granulocyte and lymphocyte counts. Lymphocytes are mainly removed by trapping, whereas granulocytes also show adhesion [11]. An increase in circulating lymphocytes may therefore saturate the filter. In contrast, the composition of the storage solution for red blood cells influences filter efficacy [12] and suggests an interaction between the filter and the red blood cells. For the residual heart–lung machine blood in our clinical setting, no data are available. It has also been suggested that the efficacy of leukocyte depletion depends on the ratio of platelets to leukocytes [13]. Our platelet counts were relatively low, which suggests that not only the circulating leukocytes saturate the filter. The leukocyte counts showed a difference between the two leukocyte removal filters, on the one hand, and the fat removal filter, on the other hand, indicating that the leukocyte removal filters were superior in this respect. The filter efficiency of the Pall RS1 and the Fresenius Biofil 02 leukocyte removal filters in this setting was about 98%. This is lower than is expected from the data for blood bank use, but is in agreement with a previous study where for residual heart–lung machine blood, a removal rate of 95–97% was found [14]. The platelet counts in the 1000-mL samples were, in all filter types, higher than in the 500-mL samples. Platelets have a higher affinity for the filter material than leukocytes [15]. Thus, it is likely that during the first part of the filtration procedure, the fibers in the filters are coated by platelet deposition. This facilitates the adherence of the leukocytes on the fibers of the filter [16]. The overall reduction in platelet counts in the LipiGuard group and in the Biofil group is in agreement with our previous findings in residual heart–lung machine blood where we found a 50% reduction in platelet counts after the filter [14]. However, platelets were almost completely removed by the RS1 filter. This is a remarkable finding, because the filter material is polyester in all three filter types. Therefore, to explain this difference, an additional coating of the filter material must have been applied. The LipiGuard filter was specifically designed to remove fat particles. It may therefore not be surprising that this filter removed fewer leukocytes than the other two filters. However, with a 50% fat removal rate, fewer fat particles were also removed than what can be removed by the other two filters. This observed percentage of fat particle reduction is in

agreement with a previous study on cardiotomy suction blood in which the LipiGuard filter removed 46% of the free fatty acids and 30% of the triglycerides [8]. Also, Ramirez et al., using an automated fat particle analysis, demonstrated a moderate efficacy of this filter in orthopedic patients [9]. Booke et al., in contrast, demonstrated with 60% fat removal a higher efficacy of this filter in a laboratory study [7]. However, they used reconstituted blood with soy oil, in contrast to the more clinical approach that we chose, which may explain this discrepancy. Fat particles are largely composed of triglycerides, which are esters of fatty acids with glycerol [17]. When triglycerides are degraded, free fatty acids are formed. Therefore, we also measured the concentration of the triglycerides and free fatty acids before and after the filter. The concentration of the free fatty acids did not increase after filtration, nor was the concentration of triglycerides reduced. This suggests that the fat particles were trapped as a whole in the filter instead of being degraded by their passage through the filter. The fat removal did not decrease in the second 500 mL of the blood in the three filter types. This suggests that the fat removal capacity of the filters was not saturated after 1000 mL of the residual blood. The clinical measurement of fat particles is difficult for several reasons. First, it requires manual processing of the samples, which is expensive and may not be feasible when larger patient groups are involved. Second, the processed samples are assessed by phase contrast microscopy, which produces semiquantitative results. Recently, an automated method was proposed to measure the circulating fat content of the blood [9]. This method is based on the assessment of plots from automated haematology cell counters and would be suitable for larger patient groups. This method has been validated and applied to blood samples from orthopedic patients with good results [9]. Therefore, we also used this method in our study with cardiac surgical patients. The concentrations of elastase, complement, and free haemoglobin in the residual blood were highly variable between the patients. This might be caused by differences in CPB time, quantity of wound suction blood, and inflammatory reaction in the individual patient [18–20]. However, the surgical procedures were equally distributed over the three groups, and there was no correlation between the CPB time and the concentrations of elastase, complement, and free haemoglobin in the residual blood. We found only a small negative correlation between the quantity of residual blood and the plasma haemoglobin concentration. These findings suggest

that the differences in elastase and complement concentrations are caused by the individual patient's reaction, and thus, may be taken into account for the assessment of filter efficiency. The elastase and free haemoglobin concentrations increased more in the LipiGuard group than in the other two groups, indicating that under these specific clinical conditions, this filter damaged leukocytes and red blood cells by mechanical forces. In contrast, the low increase in complement C5–9 in the LipiGuard group suggests good blood compatibility. However, three LipiGuard filters became blocked before 1000 mL of the blood had passed. The trapped leukocytes also cannot explain the blocking of these filters as the leukocyte counts after the filter were higher than in the leukocyte removal filters. The activation of coagulation and platelets as well as cell debris caused by haemolysis might be an explanation for the blocking of these filters. Although this study is limited by the size of the three groups, we conclude that the two leukocyte removal filters were superior, both in leukocyte and fat removal, to the specific fat removal filter. Using a leukocyte removal filter, the capacity for residual heart–lung machine, with its concomitant low haemoglobin concentration, may safely be estimated to be 1000 mL. A short passage time and good biocompatibility may determine the choice for a leukocyte filter to remove leukocytes and fat particles from residual heart–lung machine blood.

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Chapter 6

Influence of mechanical cell salvage on red blood cell aggregation, deformability, and 2,3-diphosphoglycerate in patients undergoing cardiac surgery with cardiopulmonary bypass

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Abstract

Background: Mechanical cell salvage is increasingly used during cardiac surgery. Although this procedure is considered safe, it is unknown whether it affects the red blood cell (RBC) function, especially the RBC aggregation, deformability, and the contents of 2,3-diphosphoglycerate (2,3-DPG). This study examines the following: (1) whether the cell salvage procedure influences RBC function; and (2) whether retransfusion of the salvaged blood affects RBC function in patients.

Methods: Forty patients undergoing cardiac surgery with cardiopulmonary bypass were randomly allocated to a cell saver group (n=20) or a control group (n=20). In the cell saver group, the blood aspirated from the wound area and the residual blood from the heart-lung machine were processed with a continuous-flow cell saver before retransfusion. In the control group this blood was retransfused without processing. The RBC aggregation and deformability were measured with a laser-assisted optical rotational cell analyzer and 2,3- DPG by conventional laboratory test.

Results: The cell saver procedure did not influence the RBC aggregation but significantly reduced the RBC deformability (p=0.007) and the content of RBC 2,3-DPG (p=0.032). However, in patients receiving the processed blood, their intra-operative and post-operative RBC aggregation, deformability, and 2,3-DPG content did not differ from those of the control patients. Both groups of patients had a post-operative drop of RBC function as a result of haemodilution.

Conclusions: The mechanical cell salvage procedure reduces the RBC deformability and the cell 2,3-DPG content. Retransfusion of the processed blood by cell saver does not further compromise the RBC function in patients undergoing cardiac surgery with cardiopulmonary bypass.

Introduction

Processing the salvaged autologous blood during operation with a mechanical cell salvage device (cell saver) is a well-established blood conservation method to reduce allogeneic blood transfusion during and after cardiac surgery [1-3]. Recently, the benefit of this method has been further strengthened by reports demonstrating that the cell saver is also associated with the removal of cell-derived micro-particles and a reduction of post-operative neurocognitive complications in patients undergoing cardiac surgery with cardiopulmonary bypass [4-6]. With a cell saver, the salvaged red blood cells (RBCs) aspirated from the wound area are separated from the plasma through washing and differential centrifugation, providing a high concentration of autologous RBCs to be retransfused to patients during and after operation [2, 3, 6]. Although some safety issues of this cell-saving technique have been well-addressed in the past, such as the effect of the cell processing procedure on haemostasis and complement activation [7–9], little is known about its effects on the functional state of salvaged RBCs, especially on the behavior of RBC aggregation, deformability, and their contents of 2,3-diphosphoglycerate (2,3-DPG). It has been demonstrated that RBC aggregation and deformability are affected by blood storage [10, 11]. Moreover, it is also known that the oxygen carrying capacity of the stored blood is reduced along with the drop of 2,3-DPG content in the RBCs [12, 13]. These RBC functional changes may account for the reduced oxygen transport capacity of stored blood after transfusion. If these functional changes are also apparent in the blood collected by the cell saver, it may have clinical implications because cell savers are commonly used in patients with a relatively large amount of blood loss. For these patients, oxygen carrying capacity of the circulating blood is of utmost importance.

The aim of the present study was to examine whether in vitro the cell salvage procedure influences the RBC aggregation and deformability as well as the RBC 2,3-DPG contents. Furthermore, this study was also aimed to observe whether retransfusion of the processed blood salvaged during the operation affects RBC function in patients undergoing cardiac surgery with cardiopulmonary bypass.

Patients and Methods

Patients

This study was approved by the local Institutional Review Board of the University Medical Centre Groningen. After written informed consent was obtained, 40 patients undergoing cardiopulmonary bypass (CPB) for elective coronary artery bypass grafting, single valve replacement, or a combined procedure were prospectively included in the study. Exclusion criteria were patients less than 18 years or over 80 years old and patients presenting for emergency operation. Patients were randomized according to a computer-generated table and allocated to a cell saver group (n = 20) or a control group (n = 20). In the cell saver group, both the shed blood from the wound area during operation and the residual blood from the heart-lung machine were processed with a cell saver, whereas in the control group neither the shed blood from the wound area nor the residual blood from the heart-lung machine was processed with a cell saver. In both groups, all the salvaged blood was returned to patients during and after operation.

Anaesthesia and Cardiopulmonary Bypass

Anaesthesia was induced and maintained by target controlled intravenous infusion of propofol (plasma concentration 1.5 to 2.0 µg/mL) and sufentanil (1.5 µg/kg). Pancuronium (0.1 mg/kg) was used for muscle relaxation. Ventilatory management was aimed at normocapnia throughout the operation and in the intensive care unit, with an inspiratory oxygen fraction of 0.4, a positive end-expiratory pressure of 6 cm H_2O and a tidal volume of 6 to 8 mL/kg. Bovine lung heparin (300 IU/kg) was used for anticoagulation, which was monitored by the celite activated clotting time (ACT; International Technidyne, Edison, NJ) and maintained at a level of more than 400 seconds. The extracorporeal circuit consisted of roller pumps (Stöckert, München, Germany), a hollow fiber oxygenator (Sarns Turbo; 3M, St. Paul, MN) and a standard arterial line filter (Affinity 38µ; Medtronic, Minneapolis, MN). The priming consisted of 500mL of 10% hydroxyethylstarch (Haes; Fresenius, Bad Homburg, Germany) and 1,000 mL of lactated Ringer's solution. Pump flow was adjusted to 2.4 $L/m^2/min$. Nasopharyngeal temperature during CPB was maintained at 32°C. After the termination of CPB, heparin was neutralized by protamine in a 1:1 ratio.

Clinical Procedures

In the cell saver group, the wound blood aspirated from both the pericardium and the pleural space from the time of skin incision to wound closure was collected in a cell saver reservoir (ATR120; Fresenius). Conventional cardiotomy suction was not used. The reservoir was primed with 100 mL of normal saline with 30.000 IU/L of heparin. The salvaged blood was then processed with a Continuous Auto Transfusion System cell saver (CATS; Fresenius), which was set up and operated according to the manufacturer's instructions. The residual blood in the heart-lung machine after CPB was collected in a transfusion bag, transferred to the cell saver reservoir, and processed also by the cell saver. In the control group, conventional cardiotomy suction was applied for the salvage of wound blood that was returned to the CPB circuit without cell saver processing. The residual blood in the heart-lung machine after CPB was collected in a transfusion bag and retransfused through a standard blood transfusion system. The transfusion trigger for allogeneic packed cells was according to institutional guidelines, which included a haemoglobin level of lower than 4 mmol/L during CPB and lower than 5 mmol/L in the post-operative phase.

Blood Sampling and Assessment

To study whether the cell salvage process would influence the salvaged RBCs, blood samples were taken, respectively, before cell saver processing in the reservoir and after blood processing in the transfusion bag. To study whether retransfusion of the processed blood would affect patients, blood samples were taken from the arterial line after induction of anaesthesia, at sternal wound closure, 1 hour after arrival in the intensive care unit, and on the morning of the first post-operative day. From each sample, 5 mL of the collected blood was anticoagulated with 0.1 mM ethylenediaminetetraacetic acid (EDTA) and prepared for RBC aggregation and deformability as well as for measuring the sample haematocrit. In addition, 0.5 mL of the collected blood was mixed immediately with 1.5 mL of 8% trichloroacetic acid (TCA). After centrifugation of the mixture at 1,000 g for 10 minutes, the supernatant was stored at -80°C for further analysis of 2,3-DPG and adenosine triphosphate (ATP).

Laboratory Measurements

Both the RBC aggregation and deformability were determined by a laser-assisted optical rotational cell analyzer (Mechatronics, Hoorn, the Netherlands) [14]. This machine consists of a laser diode and a thermostated bobcup measuring system. For the in vitro blood samples taken from the salvaged blood, the haematocrit level was adjusted to 40% with the hydroxyethyl starch solution similar to the priming solution for the heart-lung machine. All the blood samples taken from patients were measured without haematocrit correction. During measurement, each sample was sheared under 37°C in a concentric-cylinder system with a gap of 0.3 mm between the cylinders. The time-dependent changes of the reflection, contributed by RBC aggregation, were measured over a period of 2 minutes at a rate of 100 samples per second. The aggregation index was then calculated from the recorded digital data in a syllectogram by the software for Windows (Mechatronics, Hoorn, the Netherlands). For the measurement of deformability, the RBCs were elongated under various shear rates that lead to shear stresses from 0 to 49.84 Pa. The elongation index was used to estimate the ability of RBC deformation, which was calculated by the ratio of long and short axes of the different patterns of the deformed RBCs at a shear rate of 3.89 Pa. From the stored TCA samples, 2,3-DPG was measured with an ultraviolet test kit (Roche Diagnostics GmbH, Mannheim, Germany) and ATP was determined by a bioluminescence assay kit (Roche Diagnostics GmbH, Penzberg, Germany) following the manufacturer's instructions.

Statistics

Data processing and statistical analysis were carried out with SPSS 14.0 (SPSS, Chicago, IL). The paired t test was used to compare the difference between the samples obtained before and after cell saver processing, whereas the Student t test was performed to compare the difference between the two groups on post-operative observations. For parameters changing with time, analysis of variance with repeated measures was performed to examine the difference between the two groups. Qualitative variables were examined by the Fisher exact test. Correlation between variables was presented by the Pearson correlation coefficient. A p value of less than 0.05 was considered statistically significant.

Results

Patients and Operation Demographics

There was no significant difference between the cell saver group and the control group with regard to patient's age, gender, height, weight, and the duration of aortic cross-clamp and CPB (Table 1).

Table 1: Patient and Operation Demographics

Demographics	Cell Saver Group (n = 20)	Control Group (n= 20)	p values
Age (years)	68 ± 9	66 ± 11	0.502
Gender (M/F)	13/7	13/7	1.000
Length (cm)	174 ± 10	172 ± 9	0.447
Weight (kg)	82 ± 13	78 ± 13	0.271
CABG	18	16	0.661
VR	1	3	0.356
CABG + VR	1	1	1.000
X-clamp time (min)	53 ± 16	62 ± 19	0.282
CPB time (min)	92 ± 24	96 ± 34	0.651

Data = mean ± standard deviation. CABG = coronary artery bypass grafting; CPB = cardiopulmonary bypass; VR = valve replacement; X-clamp = aortic cross-clamp

The collected blood for cell saver processing was 1,646 ± 484 mL from the reservoir and 937 ± 275 mL from the CPB circuit in the cell saver group, yielding 561 ± 189 mL of RBC concentrates for retransfusion, whereas in the control group the 1,160 ± 459 mL residual blood from the CPB circuit was retransfused. The haematocrit level was 17 ± 6% before cell processing and 70 ± 7% after processing.

Effect of Cell Salvage Procedure on RBC Function in Vitro

Before the cell salvage procedure, the RBC aggregation index of the salvaged blood from the cell saver reservoir as 63.0 ± 5.5%, which was within the normal range. After the processing procedure, it did not change (fig 1). However, the RBC elongation index (deformability) dropped significant after the cell salvage procedure (from 0.359 ± 0.018 to 0.305 ± 0.016, p=0.007; fig 1).

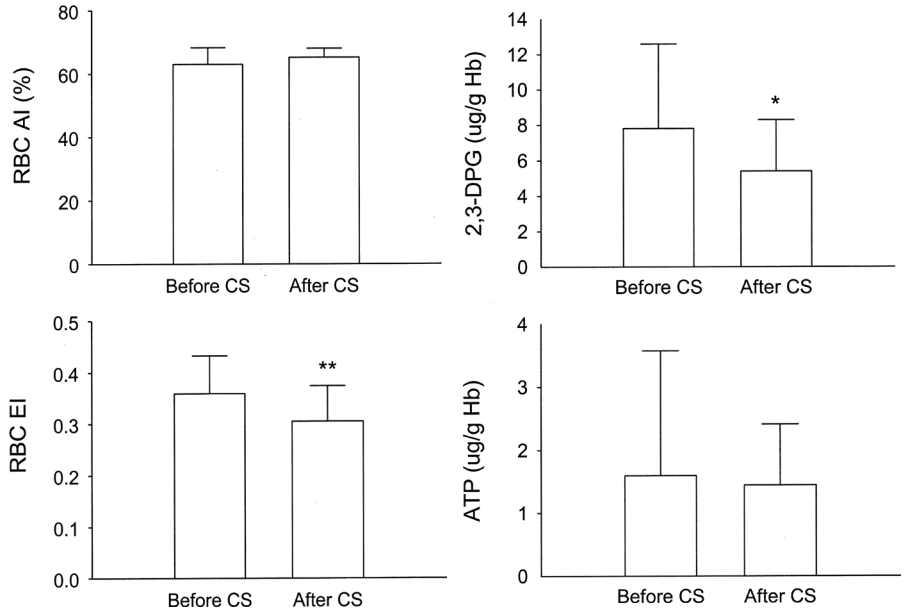

Fig 1 (left). Red blood cell (RBC) aggregation index (AI) and elongation index (EI) determined under 3.89 Pa shear stress before and after blood processing in the cell Saver (CS) group in patients undergoing cardiac surgery with cardiopulmonary bypass. Data presented are mean ± SD. (** = $p < 0.01$ compared with the previous data set by paired t test.)

Fig 2 (right). Red blood cell 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) concentrations before and after blood processing in the cell saver (CS) group in patients undergoing cardiac surgery with cardiopulmonary bypass. Data are presented in mean ± SD. (* = $p < 0.05$ compared with the previous data set by paired t test).

The 2,3-DPG concentration from the salvaged RBCs before processing was 7.80 ± 1.28 µmol/g Hb. After processing, it dropped significantly to 0.541 ± 0.77 µmol/g Hb ($p= 0.032$; fig 2). The ATP concentration from the salvaged RBCs dropped only slightly after the cell processing procedure (from 1.59 µmol/g Hb to 1.44 ± 0.24 µmol/g Hb; fig 2)

RBC Aggregation, Deformability, 2,3-DPG, and ATP in Patients

There was no significant difference between the cell saver group and control group with regard to the baseline RBC aggregation index, elongation index, 2,3-DPG, and ATP concentrations (table 2). The RBC aggregation index dropped more than a half by the end of operation and remained low at the first post-operative day in both patient

groups. Similarly, the elongation index was low at the post-operative day without difference between the two groups. The 2,3-DPG and ATP concentrations dropped significantly during operation and remained low at the post-operative day 1 in both groups (table 2). The post-operative RBC deformability and 2,3-DPG were neither associated with the amount of donor blood transfused (r = 0.129, r = 0.059) nor with the amount of processed blood retransfused (r = 0.349, r = -0.126).

Table 2: RBC aggregation, deformability, 2,3-DPG, and ATP in patients

Variables	Pre-operation	End-operation	1h-ICU	Day 1	P1	P2
RBC aggregation index (%)						
Cell saver (n=20)	50.3 ±14.3	18.6 ±11.8	19.3 ±11.2	30.6 ±13.0		
Control (n=20)	52.9 ±13.2	21.8 ±11.1	24.4 ±14.2	34.1 ±12.7	0.00	0.22
RBC elongation index (3.89 Pa)						
Cell saver (n=20)	0.356 ±0.060	0.360 ±0.061	0.350±0.059	0.347 ±0.059		
Control (n=20)	0.321 ±0.064	0.329 ±0.062	0.323 ±0.057	0.310±0.059	0.10	0.08
2,3-DPG (µmol/L)						
Cell saver (n=20)	9.2 ±3.5	5.9 ±2.1	8.0 ±4.0	6.2 ±2.6		
Control (n=20)	8.2 ±3.1	6.1 ±3.1	6.7 ±3.0	6.4 ±2.5	0.00	0.59
ATP (µmol/L)						
Cell saver (n=20)	2.9 ±1.8	1.8 ±0.8	2.5 ±2.7	1.8 ±1.1		
Control (n=20)	2.3 ±1.6	1.7 ±1.0	1.9 ±1.1	2.7 ±1.5	0.12	0.77
Haematocrit (%)						
Cell saver (n=20)	35.9 ±3.6	24.8 ±3.0	29.7 ±3.5	31.7 ±3.0		
Control (n=20)	35.1 ±3.5	23.3 ±2.7	27.0 ±3.1	28.8 ±3.3	0.00	0.02

Data are expressed as mean ± SD. 1h-ICU, 1 hour intensive care unit; 2,3-DPG, 2,3-diphosphoglycerate; ATP, adenosine triphosphate; P1, p value for interaction between group and time; P2, p value for difference between groups; RBC, red blood cells.

Post-operative Observations

Transfusion of the allogeneic RBCs during the whole intra-operative and post-operative period was 270 ± 455 mL (mean ± standard deviation) in the cell saver group and 375 ± 400 mL in the control group (p = 0.443). However, there were only 6 patients in the cell saver group versus 13 patients in the control group who received allogeneic RBC transfusion during and after the operation. Patients in the cell saver group had a significant higher haemoglobin level on the

first post-operative day than those in the control group ($p = 0.012$). Post-operative chest drainage was 460 ± 347 mL in the cell saver group and 400 ± 222 mL in the control group ($p = 0.533$). There was no statistical difference between the two patient groups with regard to the post-operative organ function and hospital stay except for a slightly higher leukocyte count in the cell saver group (table 3).

Table 3: Post-operative Observations[a]

Variables	Cell saver group (n=20)	Control group (n=20)	P Values
Leukocytes (x 10^9/L)	17.6 ± 5.9	14.3 ± 3.4	0.043
Platelets (x 10^9/L)	190 ± 44	178 ± 54	0.469
Haemoglobine (mmol/L)	6.7 ± 0.65	6.1 ± 0.69	0.012
Chest Drainage (ml/24h)	460 ± 347	400 ± 222	0.533
RBC transfusion (ml)[b]	270 ± 455	375 ± 400	0.443
RBC transfusion[c]	6/20	13/20	0.056
CPK (IU/L)	397 ± 208	496 ± 376	0.320
Creatinin (µmol/L)	76 ± 18	75 ± 29	0.840
C-reactive protein (mg/L)	30.4 ± 58.6	23.3 ± 34.1	0.644
Hospital stay (days)	9.4 ± 5.3	9.0 ± 3.7	0.811

[a] Data recorded on the first post-operative morning. [b] Total intra-operative and post-operative allogeneic blood transfusion. [c] number of patients receiving allogeneic blood transfusion. Data are expressed as mean ± SD.
CPK, creatine phosphokinase; h, hours; RBC, red blood cells.

Discussion

The safety issue of blood salvage by the cell saver device during cardiac surgery has been extensively studied in the past [7–9]. However, very little attention has been paid to the functional status of salvaged RBCs; especially to the RBC oxygen transport function, which is of particular importance in delivering oxygen to the tissue during the early post-operative period while the haemoglobin concentration is low. In the current study, we found that the elongation index, a measure of the RBC deformability, decreased significantly after the cell saver procedure. Moreover, the content of RBC 2,3-DPG, a crucial biomarker of the RBC oxygen unloading capacity, was also reduced significantly after the cell saver procedure in the salvaged blood, suggesting that the mechanical cell salvage procedure affects the RBC function of the salvaged blood.

The ability of RBCs to aggregate and to deform is one of the key determinants of blood rheology, which contributes to the maintenance of effective microcirculation and organ function [15]. In patients undergoing cardiac surgery with CPB, the RBC deformability decreases as a result of a combined effect of hypothermia, haemodilution, and mechanical stress [16, 17]. In the aspirated blood, as we observed in the current study, the RBC aggregation was within the normal range and it was not affected by the cell saver processing procedure. However, the cell saver procedure significantly reduced the RBC deformability. The mechanism by which the cell saver procedure reduces the RBC deformability is not quite clear. It is conceivable that the shear force applied during the cell saver procedure may directly affect the deformability as well as the shape of RBCs [18, 19]. However, it is uncertain whether the washing solution used during the cell saving procedure would have influenced the RBC deformability. As a routine, normal saline was used in the present study because this solution was regarded as an easy and simple solution for washing RBCs during the cell saver procedure. It remains to be determined whether a better preservation solution, as any of those used for blood storage, should be developed for the cell saver processing of the salvaged blood during cardiac surgery [20, 21].

The RBC 2,3-DPG content plays an important role in keeping the oxygen dissociation curve within normal range as 2,3-DPG lowers the affinity of haemoglobin for oxygen [22]. A reduced 2,3-DPG content functionally limits the ability of RBCs to unload oxygen in the peripheral circulation, which in turn leads to a reduced capacity of oxygen transport. In patients undergoing cardiac surgery, an early report by Schmidt and colleagues [23] revealed that the plasma level of 2,3-DPG did not change significantly in patients whose shed mediastinal blood was processed by a cell saver. However, because the content of 2,3-DPG in the salvaged RBCs was not measured in that study it was unable to judge whether or not the mechanical cell salvage procedure per se would have any detrimental effects on 2,3-DPG of the salvaged blood. In the current study, we took samples especially for this purpose and found that the 2,3-DPG content of the salvaged RBCs dropped significantly after the cell salvage procedure, which suggests that the high shear stress generated during the cell saver procedure may have resulted in the damage of cell membrane, and in turn causing 2,3-DPG depletion in the salvaged RBCs.

Despite the fact that our results have shown a reduction of RBC deformability and 2,3-DPG contents by the cell saver procedure, retransfusion of this processed blood does not seem to lead to a systemic reduction of RBC function in patients. Furthermore, with a small sample size this study does not power enough to address any connection between the changes in RBC deformability and 2,3-DPG concentration and clinical outcome such as transfusion or blood loss. As we observed in this study, patients in both the cell saver and control groups had a similar drop of RBC aggregation during operation, which is caused largely by haemodilution of the plasma factors that are necessary for the RBC aggregation [17, 24]. However, RBC deformability observed in the current study was kept stable during operation. On post-operative day one, the RBC deformability dropped in both groups, which was probably a result of a general inflammatory response known to be associated with a reduced RBC deformability [25].

In conclusion, both the RBC deformability and RBC 2,3-DPG contents dropped significantly in vitro after the cell saver procedure of the salvaged blood during cardiac surgery. For patients who received salvaged blood processed by the cell saver, their in vivo RBC function does not seem to be affected. Thus, intra-operative cell processing with the continuous-flow cell saver is safe and it does not contribute to significant RBC dysfunction after CPB. A general adverse effect of cardiopulmonary bypass on RBC aggregation, deformability, and depletion of 2,3- DPG was equally observed in patients with and without the mechanical cell saving procedure.

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Chapter 7

Summary, general discussion and future perspectives

Summary

This thesis investigated whether the use of a cell saver and the use of leukocyte depletion filters or a combination of these, could serve as a strategy to reduce allogeneic blood transfusions in adult cardiac surgery. Furthermore, we looked at the effect of cell savers and the filters on the quality of processed and retransfused blood.

Chapter 1 serves as an introduction into the different possibilities and difficulties in the current blood sparing strategies used in adult on-pump cardiac surgery, with particular attention to the standard use of cardiotomy suction.
There appears to be little to no evidence for cardiotomy suction use during CPB as a blood conserving strategy in routine cardiac surgery. Furthermore the quality of the cardiotomy suction blood is not optimal as this blood contains potential embolic substances, haemolytic blood, activated platelets and pro-inflammatory substances that can impair haemostasis and increase the inflammatory response.
The main focus of the thesis on improving or eliminating cardiotomy suction blood by filtration with leukocyte depletion filters or the use of a cell saver device is introduced. The current status of leukocyte depletion in cardiac surgery is described. Next the role of the cell saver, advocated in the most recent guidelines as a blood conserving strategy in adult on-pump cardiac surgery, is discussed. The aspects surrounding the controversies of the use of a cell saver during on-pump cardiac surgery are discussed with particular attention to initial reasons for cell saver deployment (blood sparing strategy or organ protection), the time frame used (during CPB only or more extensive) and during which type of surgery (low, intermediate or high risk).
Thus, the main questions in this thesis are whether the quality of collected and retransfused blood in cardiac surgery can be improved and whether cell savers, or the use of filters can reduce allogeneic blood transfusions in cardiac surgery.

Chapter 2 describes the multicentre, factorial designed study in which patients selected for elective on-pump cardiac surgery were randomized into four groups. One group with cell saver use, one group with cell saver and leukocyte depletion filters use and one group with only leukocyte depletion filter use to processes intra-operative shed

blood. The fourth group was a control group, with the use of standard cardiotomy suction. This study showed that the intra-operative use of a cell saver did not reduce the total number of blood products, but did reduce the percentage of patients that received allogeneic blood products. The finding of this trial has clinical implications, as transfusion of allogeneic blood products is associated with reduced long-term survival and increased morbidity. The combination of a cell saver with a leukocyte depletion filter did not result in a clinically relevant advantage for the patient nor did the novel approach to transfuse all unprocessed wound blood through a leukocyte depletion filter. The effect of preoperative anaemia, surgical procedures and red blood cell storage time on intra-operative transfusion and post-operative morbidity are also discussed. Our findings support the routine use of a cell saver during on-pump cardiac surgery.

Chapter 3 describes the study, which tries to answer the question if the quality of blood processed by a cell saver is affected when large quantities of blood are processed during adult on-pump cardiac surgery. The results of the present study demonstrate that multiple runs of the C.A.T.S. cell saver with shed intra-operative wound blood lead to a similar reduction in the concentration of the pro-inflammatory cytokine IL-6. Leukocytes were retained but haemoglobin, free haemoglobin, haematocrit and platelet concentrations were not different between the two processing runs. The study concludes that multiple runs with a cell saver device does not affect the quality of processed blood.

Chapter 4 describes the cohort study which investigates whether additional post-operative collection and processing of mediastinal shed blood with a new type of cell saver device that is also used intra-operatively could reduce the number of allogeneic blood transfusions in adult patients undergoing on-pump cardiac surgery compared to intra-operative cell salvage alone. The conclusion of the study is that continuing cell salvage beyond the operating rooms throughout a specific time in the intensive care unit did not reduce transfusion requirements further compared to intra-operative cell salvage alone. What is also shown is that the process of post-operative cell salvage with this cell saver elevates biomarkers as a side effect of haemolysis of the collected blood.

Chapter 5 elaborated on the performance of three different kinds of filters. Background for this study was that activated leukocytes and fat particles are associated with organ injury, especially the brain, after on-pump cardiac surgery. Performance of two specifically designed leukocyte depletions filters and one fat removal filter were compared in a clinical setting. This study showed that leukocyte removal filters were superior to fat removal filters in both leukocyte and fat removal. Furthermore there was a shorter passage time of blood when a leukocyte depletion filter was used.

Chapter 6 describes the study, which investigates whether the use of a cell saver device during adult on-pump cardiac surgery influences red blood cell function and if the retransfusion of this salvaged blood affects the red blood cell function in patients. What is shown is that the cell salvage procedure reduces the red blood cell deformability and the 2,3-DPG content of red blood cells, indicating a reduction in red blood cell function. But retransfusion of this processed blood by a cell saver does not further compromise the RBC function in adult patients undergoing cardiac surgery with cardiopulmonary bypass.

General discussion and conclusions

The principal results of the combined studies in the present thesis paints a picture of the use of cell saver devices and leukocyte depletion filters in adult on-pump cardiac surgery. Alas it is not a crystal clear picture.

Cell salvage of cardiotomy suction blood and blood quality aspects

The present thesis shows that collecting and processing cardiotomy blood in cardiac on-pump surgery with a cell salvage device negatively affects the red blood cell deformability and the 2,3-DPG content of collected blood. The reduction in red blood cell deformability or 2,3-DPG was also found in other studies in on pump cardiac surgical patients [1,2], but not in two other clinical trials [3,4]. One study found an increase in 2,3-DPG content when a cell saver processed residual CPB blood [3]. Two recent clinical trials, showed that retransfusion of the processed blood did not negatively affect the red blood cell function in vivo [2,4]. Although cell saving can reduce the red

blood cell function, it is important to realise that this reduction is still less then is seen with transfusing allogeneic red blood cells [4]. As the transfusing of allogeneic blood products is associated with increased morbidity and mortality in patients undergoing cardiac surgery [8,9], cell salvage remains an attractive option to reduce transfusion of allogeneic blood products in cardiac surgical procedures.
Processing large amounts of blood with a continuous device does not affect the ability of the device to remove pro-inflammatory cytokines. Processing cardiotomy blood with a cell saver might in this way reduce the systemic inflammatory associated with on-pump cardiac surgery [5-7].

Leukocyte depletion filters as blood transfusion sparing strategy

As mentioned in the introduction, the use of a leukocyte depletion filter during CPB is not recommended in the current guidelines [10]. Although leukocyte depletion is often reported as a successful method in reducing inflammation, nearly all of the clinical trials have failed to demonstrate clinical benefits on overall patient outcomes such as morbidity, mortality, and hospital stay [11-14]. What is shown in the present thesis is that these filters are effective in their task, are safe and can be used in a clinical setting. Blood retransfused with a leukocyte depletion filter has potential plasma saving effect, i.e. the plasma fraction of the retransfused blood is retained contrary to the use of a cell saver. But the use of a leukocyte depletion filter to improve the quality of the intra-operatively shed blood does not reduce transfusion requirements. We have not been able to show that using a leukocyte filter improves patient outcome compared to standard care or the use of a cell saver. Therefore we cannot recommend the use of leukocyte depletion filters in routine adult on-pump cardiac adult surgery as a blood transfusion sparing strategy.

Cell savers as blood transfusion sparing strategy

The present thesis also shows that the use of cell savers in on-pump cardiac surgery, instead of the standard care cardiotomy suction, reduced the percentage of patients transfused allogeneic blood products by 10%. However the total amount of blood products transfused was not reduced. This appears to be a paradox. The probable explanation is that the amount of red blood cell transfusions spared is offset by an

increase in the amount of FFP's or platelets transfused since these are completely eliminated during the washing process with the cell saver.

Earlier studies that primarily focussed on processing cardiotomy suction blood found that more patients in the cell saver group received FFP [15,16]. The cell saver initial main deployment was as organ protection for the brain. In our study we did not observe that more patients received FFP despite the fact that the cell saver was used during the entire operation and processed larger quantities of shed blood [17]. Other studies using cell savers in combination with cardiotomy suction had similar results as our study regarding the transfusion of FFP's [18,19].

In our study, which is the largest cell saver study to date, we found however, that if FFP was transfused to patients in cell saver groups, more FFP was transfused compared to patient in the non-cell saver group [17]. Thus, if individual patients suffer from bleeding complications the use of a cell saver may have an adverse effect, probably by the removal of plasma with its coagulation factors and platelets. So, paradoxically, in that situation cell saving in cardiac on-pump operations with large amounts of blood loss may induce a downward spiral of bleeding and increase the total amount of blood products used.

But it is still unclear if the complex cardiac surgical procedure itself or if the use of the cell saver during the procedure is responsible for the increase in haemostatic products. In our study, higher volumes of processed cell saver blood were associated with increased transfusion rates of haemostatic products [17]. With more complex procedures, more blood is collected and processed by the cell saver. As such there is an association between the different surgical procedures, cell saver use and transfusion of haemostatic components. On the other hand, when we compared the use of haemostatic products in patients with CABG, valve surgery and combined procedures, we found no significant effect on the transfusion requirements of haemostatic products whether a cell saver was used or not. This suggests that use of a cell saver per se during the various surgical procedures is not associated with more bleeding disorders. Thus, excessive intra-operative bleeding rather than the type of operation is probably the more important factor for increased haemostatic component transfusions.

Two recent studies showed that routine use of a cell saver in low risk cardiac surgery does not reduce allogeneic transfusions [20,21]. These studies either did not use the cell saver during CPB or a large proportion of the population consisted of off-pump surgery. Contradictory to these studies, two other large studies supported the view that cell saver use in low to intermediate risk surgery reduces allogeneic RBC transfusions [22,23]. One study also showed that the use of a cell saver reduced RBC transfusions in the high-risk surgery groups [22]. The current evidence available thus favours the standard use of a cell saver device to reduce the total amount of allogeneic blood product transfused compared to cardiotomy suction, also in patients with expected little blood loss. But more evidence is needed to help guide clinicians with respect to identifying cardiac surgical patients in who cell salvage might be disadvantageous.

Future directives and implications for further research

First, as mentioned above, more evidence is needed for the use of a cell saver in operations with large amounts of blood loss or extended CPB runs. To achieve this it is important that future studies record and report the volume of cardiotomy suction blood collected and retransfused. This would give the opportunity to better characterize the specific effects of cell saving versus surgical bleeding during complex surgical procedures on transfusion of haemostatic products.

Second, currently three types of cell saver are available. Those with a fixed volume bowl system (Haemonetics (Cell saver®), Sorin (Xtra®, Electa®; BRAT 2®), Medtronics (Autolog®)), with a variable volume bowl system (Haemonetics (CardioPat®; OrthoPat®)) and the continuous, non-bowl, rotary system (Fresenius, (C.A.T.S®)). There is no recent research on the quality of the blood processed by these different types of cell savers. Furthermore not all types of cell savers systems, inherent to the type of system, are suited for use in procedures with high bleeding leading to processing of larger volumes. Whether the type of cell saver system used influences the quality of the processed blood and the need for blood transfusion in cardiac surgery is a question worth investigating.

Third, in reporting cell saver studies in adult on-pump cardiac surgery it is important that all data of the cell saver and other volumes of blood collected are presented. For an objective discussion on cell saver efficacy, studies must clearly state during

which part of the surgery and CPB period the cell saver was used. It is important to note in the methods section if the cell saver was used before and after CPB, during CPB, if processing of residual CPB blood was performed and if the cell saver was used post-operatively. Then the total volume collected by the cell saver device and the volume obtained after processing (i.e. the crude extraction ratio) must be reported. This information is automatically available in all modern types of cell saving devices. To even better understand the efficacy of cell savers it would be wise to report the haematocrit of the collected and of the processed blood. The haematocrit is essential to interpret the washing process and the finished product and makes calculating the exact extraction ratio possible [24]. Initial purpose of cell saver deployment is also important to state: organ protection or blood sparing strategy.

Finally, in order to better understand blood utilization in adult cardiac surgery it is necessary to uniformly report allogeneic blood transfusions in studies in a standardized manner. Reporting the total amount of transfusions, the proportion of patients without a transfusion and how many allogeneic blood products were actually transfused per patient can help to lift the veil on the well-kept secret of blood utilization in cardiac surgery.

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Chapter 8

Nederlandse samenvatting voor de niet-ingewijden

Inleiding

Het doel van deze Nederlandse samenvatting is om diegenen die niet met dit onderwerp vertrouwd zijn, kennis te laten nemen van de inhoud van dit proefschrift getiteld: ”The role of cell savers and filters in cardiac surgery”, vrij vertaald als “De rol van cell savers en filters in de hartchirurgie”. Alvorens in te kunnen gaan op de inhoud van het proefschrift, zal ik eerst wat achtergrond informatie geven.

Voor een groot deel van de hartchirurgische operaties is het nodig om het hart van de patiënt (tijdelijk) stil te leggen. Dit geldt voor alle operaties aan de hartkleppen en voor een deel van de operaties waar omleidingen op de kransslagaderen (*CABG= coronary artery bypass grafting*) worden aangelegd.
Tijdens zo’n operatie neemt een zogenaamde hart-longmachine de bloedsomloop van de patiënt over en voorziet de vitale organen (brein, nieren, lever) van voldoende bloed en zuurstof. Het hart en de longen van de patiënt worden dus omzeild (*CPB= cardio-pulmonary bypass*). Maar om het bloed door lichaamsvreemde materialen te kunnen laten stromen is het essentieel dat tijdens de hart-longmachine periode het bloed onstolbaar (*anticoaguleren*) wordt gemaakt met een bloedverdunner (*heparine*).

Maar als het bloed niet kan stollen, kan er ook veel bloedverlies tijdens de operatie optreden. Dit verloren bloed (*shed blood*) kan zich ophopen zowel in de holte waar het hart zich in bevindt (*pericard en mediastinum*), als ook in de ruimte waar de longen zich bevinden (*pleuraholte*).
Weggooien van dit verloren bloed zou betekenen dat veel meer patiënten een bloedtransfusie zouden moeten krijgen. Bloedtransfusies worden in de hartchirurgie frequent gegeven. En hoewel deze levensreddend kunnen zijn, is ook bekend dat bloedtransfusies een negatief effect op de overleving van de patiënt kunnen hebben.

Een van de grote uitdagingen in de hartchirurgische praktijk is een manier te vinden om het verloren bloed zo veilig en eenvoudig mogelijk terug te geven aan de patiënt en tegelijk zo weinig mogelijk bloedtransfusies te geven.

Vanaf de jaren zeventig van de vorige eeuw werd het verloren bloed opgezogen en opgevangen in een reservoir (*cardiotomie reservoir*) en via de hart-longmachine weer teruggegeven aan de patiënt. Dit heet gebruik maken van cardiotomy suction. Nu blijkt het bloed dat op deze manier opgevangen en aan de patiënt terug gegeven wordt, vol te zitten met geactiveerde witte bloedcellen (*leukocyten*) en andere stoffen die de bloeddruk negatief kunnen beïnvloeden (*vaso-active substancies*) zoals vet en kleine micropropjes (*micro-thrombi*) die o.a. de bloedstolling, nieren en de hersenwerking negatief kunnen beïnvloeden. Om dit probleem van "niet-schoon bloed" te ondervangen, maar het bloed nog wel aan de patiënt terug te geven, is bedacht om een zogenaamde cell saver in te zetten. Er wordt dan geen of minder gebruik gemaakt van het cardiotomie reservoir.

Cell saving

Cell saving is een techniek waarbij het bloed dat verloren gaat tijdens een operatie op te vangen (*collect*), het te verwerken (*process*) en daarna terug te geven aan de patiënt (*retransfuse*). Het opvangen en de verwerking van het bloed gebeurt door een daarvoor speciaal ontworpen apparaat, de cell saver. Dit apparaat is in eerste instantie bedacht om bloed, verloren tijdens niet-hartchirurgische operaties (zonder gebruik van de hart-long machine) terug te geven. Het bloed wordt onstolbaar gemaakt met heparine en opgevangen in een reservoir. Vervolgens wordt het bloed met hoge snelheid gecentrifugeerd en geconcentreerd. Dan wordt het gewassen met een fysiologische zoutoplossing. Door het centrifugeren blijven alleen de rode bloedcellen over die teruggegeven kunnen worden aan de patiënt. Het plasma en de bloedplaatjes, dus de bloedstolling producten, gaan daarbij verloren. Door het wassen worden de laatste resten van leukocyten en plaatjes, de micro-thrombi vet, de heparine en vaso-active substancies verwijderd.

Filters

Tijdens de hart-longmachine periode worden er normaliter al filters gebruikt zodat de micro-thrombi en vetdruppels niet in de bloedsomloop van de patiënt terecht komen. Er zijn de afgelopen jaren verschillende nieuwe filters ontwikkeld die specifiek leukocyten kunnen wegvangen (*filtreren*).

De duur van de hart-longmachineondersteuning aan een patiënt kan een activatie geven van leukocyten. Vooral het bloed dat zich een tijd in de holtes van hart en longen heeft bevonden kan hieraan bijdragen.
Geactiveerde leukocyten scheiden stoffen af die een algemene ontstekingsreactie kunnen doen ontstaan (*pro-inflammatoire reactie*). Deze reactie is, hoewel bij ziekte gewenst, tijdens en na hartchirurgie juist ongewenst. Deze ontstekingsreactie kan er namelijk voor zorgen dat het bloed van een patiënt minder goed stolt na de operatie en dat de patiënt veel vocht of medicatie nodig heeft om de bloeddruk voldoende hoog te houden.
Deze ongewenste ontstekingsreactie van de geactiveerde leukocyten kan (deels) voorkomen worden door het opgevangen bloed eerst door een leukocyten filter te laten lopen. De geactiveerde leukocyten worden dan in het filter gevangen en kunnen niet aan de patiënt teruggegeven worden. Het plasma en de bloedplaatjes, dus de bloedstolling producten, gaan hierbij niet verloren. Het is alleen niet bekend of deze techniek het aantal bloedtransfusies na hartchirurgie kan verminderen.

Onderzoeksvragen
In dit proefschrift wordt daarom de rol van cell savers, filters en de combinatie daarvan op het verminderen van bloedtransfusies in de hartchirurgie onderzocht. Daarnaast wordt gekeken naar het effect van het gebruik van een cell saver of filter op de kwaliteit van het teruggegeven bloed.

Hoofdstuk 1
Dit hoofdstuk is een introductie van het proefschrift. Het beschrijft de verschillende mogelijkheden en problemen van de huidige bloedbesparende technieken in de hartchirurgie. Bloedbesparing is gewenst omdat, hoewel soms levensreddend, bloedtransfusies geassocieerd worden met een verhoogde morbiditeit en mortaliteit. Het gebruik van een cardiotomie reservoir wordt besproken. Het voordeel van een cardiotomie reservoir lijkt, hoewel niet bewezen, dat er minder bloedtransfusies gegeven worden. De nadelen van een cardiotomie reservoir zijn onder andere het teruggeven aan de patiënt van “niet-schoon bloed”.

De mogelijkheden van een filter als bloedtransfusie besparende strategie bij het gebruik van een cardiotomie reservoir worden besproken. Vervolgens wordt de rol van cell savers beschreven bij het verbeteren van de kwaliteit van het "cardiotomie-bloed" en het verminderen van bloedtransfusies. Daarnaast wordt de bestaande literatuur over bloedbesparing door het gebruik van de cell saver in de hartchirurgie uitgebreid besproken. Hoewel de huidige consensus en aanbevelingen zijn dat een cell saver voordelig is om te gebruiken tijdens cardio-chirurgische operaties met een hart-long machine zijn er nog altijd aspecten die verduidelijking behoeven.

Hoofdstuk 2

Dit hoofdstuk beschrijft de multicenter (deelname van meerdere ziekenhuizen) en factoriaal (2 interventies worden zowel elk apart als in combinatie bestudeerd tegenover een groep waarbij geen interventie wordt toegepast) opgezette studie waarbij patiënten bij geplande cardio-chirurgische operaties met een hart-long machine gerandomiseerd verdeeld werden in vier groepen. Een groep waarbij een cell saver werd ingezet, een groep waarbij een cell saver met leukocytenfilter werd ingezet, een groep waarbij alleen het leukocyten-depletiefilter werd gebruikt om verloren bloed te bewerken en terug te geven. In de vierde groep werd de standaardbehandeling met cardiotomie-suction gebruikt.

Wij tonen aan dat het gebruik van een cell saver tijdens de hartchirurgische operatie geen verlaging geeft van het totale aantal toegediende bloedproducten tijdens de ziekenhuis opname, maar dat het percentage patiënten die een bloedtransfusie kregen wel werd verlaagd. De bevindingen van dit onderzoek heeft klinische consequenties, want de transfusie van bloedproducten wordt geassocieerd met een vermindering van de lange termijn overleving en een verhoogde morbiditeit.

De combinatie van een cell saver met het gebruik van een leukocyten-depletiefilter resulteerde niet in een klinisch relevant voordeel voor de patiënt evenmin als het filtreren van verloren bloed door een leukocyten-depletiefilter alleen.

Onze bevindingen ondersteunen het gebruik van een cell saver tijdens hartchirurgische operaties met een hart-long machine.

Tenslotte worden het effect van pre-operatieve bloedarmoede, hartchirurgische operaties en de bewaartijd van rode bloedcellen op intra-operatieve bloedtransfusies en post-operatieve mortaliteit besproken.

Hoofdstuk 3

Dit hoofdstuk beschrijft de klinische studie waarin wij onderzocht hebben of de kwaliteit van bloed verwerkt door een cell saver constant blijft. Afhankelijk van de hoeveelheid bloed die opgevangen wordt, kan een cell saver meerder malen tijdens dezelfde operatie ingezet worden (*multiple runs*).

In deze studie hebben wij ook gekeken of opeenvolgende runs van een cell saver tijdens hart-chirurgische operaties met een hart-longmachine eenzelfde daling in de concentratie van het pro-inflammatoire cytokine IL-6 laat zien als een enkele processing run.

Wat we vonden was dat met multiple runs de daling van de concentratie van IL-6 constant bleef. Hemoglobine, vrij hemoglobine, en hematrocriet bleken ook gelijk te blijven. Verder bleek dat met multiple runs er een concentratie effect van leukocyten optrad en de bloedplaatjes niet verschillend waren tussen de twee runs. Wij concluderen dan ook dat meerdere runs van een cell saver de kwaliteit van het bloed niet negatief beïnvloeden.

Hoofdstuk 4

Dit hoofdstuk beschrijft de studie waarin wij als onderzoeksgroep gekeken hebben naar het gebruik van een nieuw soort cell saver. Deze nieuwe cell saver kan behalve tijdens de hartchirurgische operatie, ook gebruikt worden om bloed verloren na de operatie op te vangen en na verwerking aan de patiënt terug te geven (*post-operatieve autotransfusie*).

De verwachting was dat er een vermindering van het aantal bloedtransfusies zou kunnen worden bereikt als bloed verloren tijdens en ook na de hartchirurgische operatie met hart-longmachine aan de patiënt teruggegeven zou worden. Om te kijken of dit inderdaad bereikt is, vergeleken we twee groepen. Een groep waarbij de cell saver alleen tijdens de operatie gebruikt werd en een groep waarbij het tijdens en na de operatie verloren bloed teruggegeven werd door de nieuwe cell saver. Wij lieten zien dat het gebruiken van de cell saver ook gedurende een specifieke tijd na de operatie op de intensive care geen verdere vermindering van bloedtransfusies geeft.

Wat wij ook zagen is dat het post-operatief bloed opvangen en verwerken een toename geeft van een biologische marker die ook gebruikt wordt als indicator

van hartspier schade, zonder dat er daadwerkelijke schade is opgetreden. Dit komt waarschijnlijk door hemolyse van het opgevangen bloed.

Hoofdstuk 5

Dit hoofdstuk beschrijft de prestaties van drie verschillende filters. Deze filters worden in de cardio-chirurgische praktijk gebruikt om geactiveerde leukocyten en vetdeeltjes uit bloed te halen/filteren dat teruggegeven wordt aan de patiënt tijdens een hartoperatie. De prestaties van twee specifieke voor leukocyten-depletie ontworpen filters en een vetfilter worden in een klinische situatie met elkaar vergeleken. Gekeken werd naar de doorlooptijd van het bloed, het aantal leukocyten en bloedplaatjes en de concentratie van totaal hemoglobine, triglyceride en vrije vetzuur in het bloed na het filtreren. Verder keken we naar vrij hemoglobine, plasma elastase (maat voor witte bloed cel activatie) en complement C5–9 (maat voor activatie stolling).
Deze studie laat zien dat leukocyten-filters beter zijn in het filtreren van bloed ten aanzien van vet en witte bloedcellen in vergelijking met een vetfilter. Verder lieten wij zien dat het bloed sneller door een leukocyten-filter loopt, wat van klinisch belang kan zijn.

Hoofdstuk 6

Dit hoofdstuk gaat over het klinisch onderzoek dat we hebben uitgevoerd om te kijken of het gebruik van een cell saver tijdens hart-chirurgische operaties met een hart-long machine de rode bloedcel-functie negatief beïnvloedt. Patiënten die een hart-chirurgische operatie ondergingen, werden gerandomiseerd in een groep waarbij verloren bloed met een cell saver werd bewerkt en een groep waarbij het bloed zonder cell saver werd teruggegeven.
In beide groepen werd er gekeken naar afgeleide parameters die informatie geven over de zuurstof-transportfunctie van de rode bloedcel. Dit zijn onder andere de vervormbaarheid en het 2,3-DPG gehalte. Wij concludeerden dat de vervormbaarheid en het 2,3-DPG gehalte van de rode bloedcellen verminderd wordt in vitro door het gebruik van een cell saver. Maar dat re-transfusie van dit bloed aan de patiënt in vivo de rode bloedcel-functie niet verder verslechterd. Verder lieten wij zien dat het gebruik van de hart-longmachine een negatief effect heeft op de klontering van de rode bloedcel, de vervormbaarheid en het 2,3-DPG gehalte.

Hoofdstuk 7

In dit hoofdstuk geven we een samenvatting van de beschreven studies en komen de conclusies uit het proefschrift naar voren.

De eerste conclusie uit het proefschrift is dat het gebruik van een cell saver een nadelig effect kan hebben op de kwaliteit van de opgevangen rode bloedcellen, maar dat dit effect niet terug te vinden is in het bloed van de patiënt. Verder blijkt dat de kwaliteit van bloed, gemeten door afname van de pro-inflammatoire cytokine IL-6, door een cell saver niet afneemt als er grotere hoeveelheden bloed opgevangen, gewassen en geconcentreerd wordt.

De tweede conclusie uit het proefschrift is dat het gebruik van een filter als bloedbesparende techniek bij hartchirurgie met behulp van een hart-longmachine niet is aan te raden.

De derde conclusie is dat het gebruik van een cell saver als bloedbesparende techniek tijdens hartchirurgie met een hart-longmachine wel kan worden aangeraden. Zelfs bij verwacht weinig bloedverlies omdat het gebruik van een cell saver de hoeveelheid patiënten die een bloedtransfusie krijgt, doet verminderen. Maar mogelijk draagt het gebruik van de cell saver eraan bij dat de totale hoeveelheid toegediende bloedproducten niet vermindert bij operaties met veel bloedverlies, door het verlies aan plasma en bloedplaatjes.

Aandachtspunten voor verder onderzoek zijn dat er nader gekeken moet worden naar het nut ten aanzien van het inzetten van een cell saver bij hartoperaties met een hart-longmachine met verwacht veel bloedverlies en naar de invloed van verschillende cell savers op het bloedbesparende effect.

Verder is het van belang dat het cell saver-gebruik op een uniforme en complete wijze in komende studies beschreven wordt. Als laatste is het voor toekomstige studies van belang dat het gebruik van bloedproducten op een uniforme wijze wordt weergegeven.

Chapter 9

Curriculum vitae and list of publications

Curriculum vitae

Jan Wytze Vermeijden werd geboren op 9 december 1972 in Haarlem. Hij groeide op in verschillende plaatsen in Nederland, kort in de Verenigde staten, en vanaf 1984 in België. Na het behalen van zijn eindexamen in 1991 aan de Europese School van Brussel II in Woluwe, België, viel zijn keus op de studie geneeskunde. Na hiervoor te zijn uitgeloot, besloot hij voor een jaar psychologie aan de Rijksuniversiteit Utrecht te studeren. In 1992, na een inhaalcursus VWO natuurkunde, werd hij ingeloot voor de studie geneeskunde te Utrecht. Na een studie-onderbreking van 9 maanden in 1993, behaalde hij in 2000 zijn artsenbul om vervolgens als AGNIO intensive care in het Catharina ziekenhuis in Eindhoven te werken. In 2002 begon hij, na een jaar thorax intensive care in het UMCG, aan zijn opleiding tot anesthesioloog aldaar. Tijdens deze periode werd zijn interesse in het verrichten van wetenschappelijk onderzoek verder gestimuleerd door samenwerking met Hans de Vries. Deze samenwerking legde het fundament voor de huidige promotie. Na het voltooien van zijn opleiding tot anesthesioloog in 2007 werd hij fellow intensive care in het UMCG om daar in maart 2008 zijn opleiding tot anesthesioloog-intensivist definitief af te ronden.

In 2008 trad hij toe tot de maatschap anesthesiologie in het Medisch Spectrum Twente in Enschede en werkte hij als anesthesioloog-intensivist op het Thorax Centrum Twente. In 2010 verliet hij de maatschap anesthesiologie en stapte over naar de toen nog op te richten maatschap intensive care. Om zich vanaf dat moment volledig op de intensive care te richten.

Wytze vormt sinds 1993 een koppel met Floor Wilke. Samen hebben ze drie fantastische kinderen, een hond, een kat en een vis.

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Chapter 10

Dankwoord

“Eindelijk!” Anders kan ik het niet zeggen. Het is een lang traject geweest. Promoveren na je opleiding, als medisch specialist, buiten je opleidingsziekenhuis en met een vol en druk gezinsleven is niet makkelijk. Promoveren in een druk perifeer ziekenhuis voelde als een bijna continue test van het Peter’s Principle: “did I rise to my own level of incompetence?”. Maar het is volbracht. Eindelijk langs mijn studeerkamer kunnen lopen zonder schuldgevoel, eindelijk verlost van de continue druk en eindelijk kunnen zeggen: “yes, het is eindelijk volbracht.”

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